PATENT
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APPLICATION FOR UNITED STATES LETTERS PATENT

STEREOSELECTIVE SYNTHESIS OF 24-HYDROXYLATED COMPOUNDS USEFUL FOR THE PREPARATION OF AMINOSTEROLS, VITAMIN D ANALOGS, AND OTHER COMPOUNDS

Inventors:

William A. Kinney
Steven Jones
Xuehai Zhang
Meena N. Rao
Michel Bulliard
Harold Meckler
Nancy Lee

CROSS-REFERENCE TO RELATED APPLICATION

This application claims priority benefits under 35 U.S.C. § 119 based on U.S. Provisional Patent Application No. 60/032,378, which was filed December 6, 1996. This provisional application is entirely incorporated herein by reference.

BACKGROUND OF THE INVENTION

Several aminosterol compositions have been isolated from the liver of the dogfish shark, *Squalus acanthias*. One important aminosterol is squalamine (3β-(N-[3-aminopropyl]-1,4-butanediamine)-7α,24ζ-dihydroxy-5α-cholestane 24-sulfate), the chemical structure of which is shown in Fig. 1. This aminosterol, which includes a sulfate group at the C-24 position, is the subject of U.S. Patent No. 5,192,756 to Zasloff, et al., which patent is entirely incorporated herein by reference. This patent describes antibiotic properties of squalamine.

Since the discovery of squalamine, however, several other interesting properties of this compound have been discovered. For example, as described in U.S. Patent Appl. Nos. 08/416,883 (filed April 20, 1995) and 08/478,763 (filed June 7, 1995), squalamine may function as an antiangiogenic agent. These patent applications are entirely incorporated herein by reference. Additional uses of squalamine (e.g., as an agent for inhibiting NHE3 and as an agent for inhibiting endothelial cell growth) are disclosed in U.S. Patent Appl. No. 08/474,799 (filed June 7, 1995) and U.S. Patent Appl. No. 08/840,706 (filed April 25, 1997). These patent applications also are entirely incorporated herein by reference.

Methods for synthesizing squalamine have been devised, such as the methods described in WO 94/19366 (published September 1, 1994). This PCT publication is entirely incorporated herein by reference. This PCT application relates to U.S. Patent Appl. No. 08/023,347, which application also is entirely incorporated herein by reference. Additionally, U.S. Patent Appl. No. 08/474,799 also discloses squalamine isolation and synthesis techniques.

Stemming from the discovery of squalamine, other aminosterols have been discovered in the dogfish shark liver and have been investigated. One important aminosterol that has been isolated and identified has the structure shown in Fig. 2. In this application, the compound having the structure shown in Fig. 2 will be referred to as "compound 1436" or simply "1436." This compound has the general molecular formula $C_{37}H_{72}N_4O_5S$ and a calculated molecular weight of 684.53017. Like squalamine, this aminosterol also has a sulfate group at the C-24 position.

Compound 1436 previously has been described in U.S. Patent Appl. Nos. 08/483,057 and 08/487,443, each filed June 7, 1995. Each of these U.S. patent applications is entirely incorporated herein by reference. As further described in these patent applications, compound 1436 has a variety of interesting properties. For example, compound 1436 inhibits human T-lymphocyte proliferation, as well as the proliferation of a wide variety of other cells and tissues. Additional uses of compound 1436 are disclosed in U.S. Provisional Patent Appl. No. 60/017,627 (filed May 17, 1996) and the subsequently filed U.S. Patent Appl. Nos. 08/857,288 filed May 16, 1997 and 08/962,290 filed October 31, 1997. These patent applications also are entirely incorporated herein by reference.

U.S. Patent Appl. Nos. 08/483,057 and 08/487,443 describe the structure of compound 1436 as well as processes for synthesizing and isolating the compound. For example, as described in these applications, compound 1436 can be prepared from a squalamine starting material.

When squalamine is isolated from dogfish shark liver, the sulfate group is located at the C-24 position, and there is no difficulty in providing the sulfate group at this location in a stereoselective manner. Likewise, when compound 1436 is derived from a squalamine starting material, the sulfate group already is located at the C-24 position, and therefore, there is no difficulty in obtaining a stereoselective structure at the C-24 position.

Difficulties have been encountered, however, when attempting to provide a process for synthesizing squalamine or compound 1436 from commercially available starting materials (i.e., not from shark liver isolates). These difficulties include low overall yields of the desired steroid product, because many steps are involved in the synthesis process. Additional difficulties are encountered in providing a sulfate group at the C-24 position. Particularly, it is difficult to provide the sulfate group at the C-24 position in a highly stereoselective orientation. See, for example, Pechulis, et al., "Synthesis of 24ξ - Squalamine, an Anti-Infective Steroidal Polyamine," *J. Org. Chem.*, 1995, Vol. 60, pp. 5121-5126; and Moriarty, et al., "Synthesis of Squalamine. A Steroidal Antibiotic from the Shark," *Tetrahedron Letters*, Vol. 35, No. 44, (1994), pp. 8103-8106. These articles each are entirely incorporated herein by reference. This invention seeks to overcome those difficulties in synthesizing squalamine and compound 1436.

Squalamine and compound 1436 are not the only compounds of interest that include a specified substituent, in a stereospecific orientation, at the C-24 position. For example, the above-noted patent applications describe many different aminosterol compounds that have various C-24 substituents. As another steroid example, cerebrosterol, includes a hydroxyl group in an *S*-orientation at the C-24 position. MC 903, a 1, 24-dihydroxyvitamin D analogue, also includes a hydroxyl group in an *S*-orientation at the 24 position. 1α, 24(R)-dihydroxyvitamin D_3 includes a hydroxyl group in an R-orientation at the 24 position. The chemical structures for cerebrosterol, MC 903 and 1α, 24(R)-dihydroxyvitamin D_3 are shown in Figs. 3A, 3B and 3C, respectively.

Because of the importance of squalamine, compound 1436, other aminosterols, 24*R* and 24*S*-hydroxylated steroids and vitamin-D_3 metabolites, there has been considerable interest in preparing compounds with a single stereospecific orientation at the C-24 position. Processes for producing squalamine and compound 1436 are described in the patent documents noted above. These processes, while effective in producing squalamine and compound 1436, do not enable large scale production of the desired aminosterol materials because relatively low yields are realized by these processes.

Other researchers have developed processes for stereoselectively producing cerebrosterol, MC 903, and 1α, 24(R)-dihydroxyvitamin D_3. A process for producing cerebrosterol is described in Koch, *et al.*, "A Stereoselective Synthesis and a Convenient Synthesis of Optically Pure (24R)- and (24S)-24 hydroxycholesterols," *Bulletin de la Société Chimique de France*, **1983**, (No. 7-8), Vol. II, pp. 189-194. A process for producing MC 903 is

described in Calverley, "Synthesis of MC 903, a Biologically Active Vitamin D Metabolite Analogue," *Tetrahedron*, **1987**, Vol. 43, No. 20, pp. 4609-4619. A process for producing 1α, 24(*R*)-dihydroxyvitamin D_3 is described in Okamoto, *et al.* "Asymmetric Isopropylation of Steroidal 24-Aldehydes for the Synthesis of 24(*R*)-Hydroxycholesterol, *Tetrahedron: Asymmetry*, **1995**, Vol. 6, No. 3, pp. 767-778. These articles each are entirely incorporated by reference.

One approach, as described in the above-noted articles, has been to reduce a 22-ene-24-one system in a stereoselective manner. This scheme is illustrated in Fig. 4A. The 22-ene-24-one system (material **B** from Fig. 4A) can be produced in a single step from the corresponding 22-aldehyde (material **A**) using an appropriate Wittig reagent (prepared in 2 steps). Therefore, if this reduction procedure was stereoselective, this would be a convenient two step procedure for preparing chiral C-24 alcohols (material **C**).

Unfortunately, this reaction is not stereospecific. Calverley described attempts to reduce a vitamin-D_3-22-ene-24-one with sodium borohydride and cerium (III) chloride; however, he achieved only a 38:61 ratio of the desired 24*S* product to the undesired 24*R* allylic alcohol. In the process of Calverley, the product had to be purified by chromatography and recrystallization to separate the 24*R* product from the desired 24*S* product. The 24*S* and 24*R* allylic alcohols can be very difficult to separate. Thus, this chemical process is not suitable for use on a large scale.

Koch, using a similar scheme to that described above, did not fare much better in producing a stereospecific 24*S* product. In producing cerebrosterol, Koch demonstrated that

lithium aluminum hydride, even substituted with chiral compounds, reduced a cholest-22-ene-24-one system **B** (Fig. 4A) in a ratio of 1:2 (24*S* to 24*R* allylic alcohols **C**).

Using a different reaction scheme, as illustrated in Fig. 4B, Koch also showed that the reduction of a cholest-22-yne-24-one system (material **D**), followed by partial reduction of the triple bond, gave only modest selectivity for the 24*S* stereoisomer, using a lithium aluminum hydride reducing reagent. A 2:1 ratio of 24*S* to 24*R* allylic alcohols **C** was obtained in this reaction scheme.

There has been one successful reduction of a related 25-ene-24-one system using Noyori's 2,2'-dihydroxy-1,1'-binaphthyl lithium aluminum hydride reagent at -90°C to give 95:5 selectivity for the 24*R*-alcohol. The procedure is described in Ishiguro, *et al.* "Stereoselective Introduction of Hydroxy-Groups into the 24-, 25-, and 26-Positions of the Cholesterol Side Chain," *J. C. S. Chem. Comm.*, **1981**, pp. 115-117, which article is entirely incorporated herein by reference. The 25-ene-24-one intermediate material (producible in four steps) is less readily accessible than the 22-ene-24-one system (producible in one step). This factor decreases the desirability of this route. Additionally, the low temperature required for the chiral reduction also detracts from the commercial practicality of this method.

One lengthy approach has been to alkylate a C-22 sulfone with a chiral epoxide. Because of the poor selectivity obtained from chiral reductions of material **B** shown above (Fig. 4A), Koch found this six step procedure from the 22-aldehyde preferable, using a reagent based on valine (producible in four steps). Overall, 10 steps were required by this process to do what one could do in four steps, if a stereoselective reduction technique was available.

Finally, Okamoto successfully used chiral β-amino alcohol catalyzed addition of diisopropylzinc to steroidal 24-aldehydes to provide 24*R*-hydroxycholesterols in good yields with high diastereoselectivities (97:3). Again, however, overall, more steps are required to yield the desired pure alcohol.

SUMMARY OF THE INVENTION

It is an object of this invention to overcome the various problems and disadvantages described above. The process of the invention stereoselectively reduces an unsaturated alkyl ketone substituent. The alkyl ketone is attached to a fused ring base, such as a steroid ring base or a vitamin D_3 analog ring base. In the process of the invention, the unsaturated alkyl ketone reacts with a chiral oxazaborolidine reagent to stereoselectively reduce the unsaturated alkyl ketone to an unsaturated alkyl alcohol. The unsaturated alkyl alcohol can be further reduced to produce a saturated alkyl alcohol.

In the invention, the chiral oxazaborolidine reagent is preferably at least one member selected from the group of compounds illustrated in Figs. 13A through 13D (compounds 11, 12, 13, and 14). These illustrated compounds, borane complexes of Me-CBS and Bu-CBS, will be described below. "CBS" stands for "Corey-Bakshi-Shibata" reagents, which will be described in more detail below.

One group of starting materials on which the process of this invention can be used are compounds that include an alkeneone substituent attached to a fused ring base. A specific example is a compound having a 22-ene-24-one substituent on a steroid ring base. In this

instance, the alkeneone material reacts with an appropriate chiral oxazaborolidine reagent to stereoselectively reduce the alkeneone to an allylic alcohol. The allylic alcohol can be further reduced to provide a hydroxylated, saturated alkane side chain from the fused ring base.

For this embodiment of the invention, the alkeneone substituent can be produced in any suitable manner. For example, the 22-ene-24-one alkeneone material mentioned above can be produced by reacting a C-22 aldehyde substituent (on a steroid ring base) with a Wittig reagent.

A second group of compounds on which the process of this invention can be used are alkyneone compounds, such as compounds including a 22-yne-24-one substituent on a steroid ring base. In this method, the alkyneone reacts with a chiral oxazaborolidine reagent to stereoselectively reduce the alkyneone to a propargyllic alcohol. If desired, in this method, the propargyllic alcohol can be further reduced to a hydroxylated, saturated alkane.

The 22-yne-24-one starting material in this embodiment of the invention can be produced from a C-22 aldehyde on a steroid ring base. This aldehyde starting material first reacts to produce a 22-alkyne substituent on the steroid ring base, and then the 22-alkyne substituent reacts with a lithium containing reagent and anhydride to produce the 22-yne-24-one substituent on the steroid ring base.

Preferably, the process of the invention produces at least 90% of the desired isomer. Selectivity of greater than 95% is particularly preferred, and greater than 97% is most advantageous. Greater than 97% of the desired isomer corresponds to greater than 94% “de” (diastereomeric excess, which is calculated as 97% - 3% = 94%).

The invention also relates to various intermediates that are produced in the process of the invention. These intermediates are particularly useful in producing squalamine, compound 1436, or other desired aminosterols.

BRIEF DESCRIPTION OF THE DRAWINGS

Advantageous aspects of the invention will be evident from the following detailed description which should be considered in conjunction with the attached drawings, wherein:

Fig. 1 illustrates the chemical structure of squalamine;

Fig. 2 illustrates the chemical structure of compound 1436;

Fig. 3A illustrates the chemical structure of cerebrosterol;

Fig. 3B illustrates the chemical structure of MC 903;

Fig. 3C illustrates the chemical structure of 1α, 24(*R*)-dihydroxyvitamin D_3;

Fig. 4A generally illustrates a first reaction mechanism for producing an unsaturated alcohol from an aldehyde starting material;

Fig. 4B generally illustrates a second reaction mechanism for producing an unsaturated alcohol from an aldehyde starting material;

Fig. 5 illustrates reaction mechanisms generally showing the process of the invention;

Fig. 6A shows a first substituent group that can be included on the fused ring starting material in the process of the invention;

Fig. 6B shows a second substituent group that can be included on the fused ring starting material in the process of the invention;

Fig. 7 illustrates a reaction scheme for producing an alkeneone from an aldehyde in the process of the invention;

Fig. 8 illustrates a reaction scheme for converting an alkyneone starting material to a saturated alcohol in a stereoselective orientation in the process of the invention;

Fig. 9 illustrates a reaction scheme for producing the alkyneone starting material used in the process shown in Fig. 8;

Fig. 10A is a general reaction scheme for producing an aldehyde material for use in the process of the invention from a commercially available starting material;

Fig. 10B shows a reaction scheme for producing an aminosterol from the aldehyde material produced in the process of Fig. 10A;

Fig. 10C shows a reaction scheme for using the aldehyde material produced in the process of Fig. 10A to make a stereospecific alcohol;

Fig. 11A illustrates the chemical structure of an intermediate used in making stereoselective 24-hydroxylated steroids;

Fig. 11B illustrates a reaction mechanism for producing stereoselective 24-hydroxylated steroids using the intermediate of Fig. 11A;

Fig. 12 illustrates a reaction mechanism for producing the intermediate of Fig. 11A as well as stereoselective 24-hydroxylated steroids;

Figs. 13A and 13B are oxazaborolidine-borane complexes used to produce *R*-allylic alcohols from enones;

Figs. 13C and 13D are oxazaborolidine-borane complexes used to produce *S*-allylic alcohols from enones;

Fig. 14 illustrates a reaction mechanism for producing a specific stereospecific 24-hydroxylated steroid;

Fig. 15A illustrates a reaction mechanism for producing steroid 29, which can be used for producing squalamine and compound 1436;

Fig. 15B illustrates a reaction mechanism for producing compound 1436 from steroid 29;

Fig. 16A illustrates a general reaction scheme for producing a class of steroids including steroid 29;

Fig. 16B illustrates a specific reaction mechanism for producing steroid 29 based on the general mechanism shown in Fig. 16A; and

Fig. 17 illustrates a reaction mechanism for producing squalamine from steroid 37.

DETAILED DESCRIPTION OF THE INVENTION

As described above, there is a need in the art for a stereoselective process for synthesizing compounds having a 24-hydroxylated steroid or vitamin D analog structure. The stereospecific 24-hydroxylated compound can itself be the desired final compound (e.g., in producing cerebrosterol, MC 903, or 1α, 24(*R*)-dihydroxyvitamin D_3). Alternatively, the process of the invention can be used to make a hydroxylated intermediate composition that can be further modified to produce the desired final product. For example, the process according to the invention can be used to produce stereospecific intermediates that can be further modified to

synthesize squalamine, compound 1436, other useful aminosterols, or steroids. Such compounds include stereospecific groups (e.g., sulfate groups in an *R* orientation for squalamine and compound 1436) at the 24 position.

The method according to the invention includes processes for stereoselectively reducing cholest-22-ene-24-one and cholest-22-yne-24-one systems. In one process of the invention, Corey's (*R*)-methyl oxazaborolidine reagent reacts with a cholest-22-ene-24-one material to produce the desired 24*S*-allylic alcohol in good yield and high stereoselectivity (>98:2). This reaction scheme is scalable to kilogram quantities because of the moderate temperature used (-20°C) and because of the reasonable cost of the chiral oxazaborlidine reagent. The cholest-22-ene-24-one material is easily prepared from the 22-aldehyde material, in high yield, without the need for a chromatography step. Accordingly, the method according to the invention is very practical in that it provides the chiral alcohol in a few number of steps (two), with procedures that are easily scalable to large quantities.

In an alternative method according to the invention, the cholest-22-yne-24-one system is reduced stereoselectively with an (*S*)-methyl oxazaborolidine borane complex. The cholest-22-yne-24-one material (an acetylenic ketone) can be prepared from an aldehyde material in two convenient steps, thereby making this a commercially practical method. Other non-steroidal propargyl ketones have been reduced selectively using this reagent, as described in Parker, K.A. *et al.* "Asymmetric Reduction. A Convenient Method for the Reduction of Alkynyl Ketones," *J. Org. Chem.*, **1996**, Vol. 61, pp. 3214-3217. This article is entirely incorporated herein by reference.

The methods according to the invention will be described generally below, in conjunction with Figs. 5-9. The process of the invention can be used to make any material having the general fused ring base structure shown as compound **C** in Fig. 5 (e.g., any compound having a six carbon ring that shares two carbon atoms with an attached five carbon ring).

Generally, the process according to the invention relates to a method for stereoselectively reducing an unsaturated alkyl ketone substituent attached to a fused ring base. The method is generally illustrated in Fig. 5. In this method, the starting material is an unsaturated alkyl ketone **C**, attached to a fused ring base. The unsaturated bond between the 22 and 23 carbons can be a double bond or a triple bond. This is illustrated in Fig. 5 using a dashed line for the triple bond.

The fused ring, as shown in Fig. 5, may have substituent groups R1, R2, and R3. In the illustrated formula, R1 and R2 together can be, for example, two fused six membered rings **J**, forming a steroid fused ring base, as shown in Fig. 6A. This steroid ring base can include appropriate substitutent groups (e.g., alkyl groups, hydroxyl groups, amine chains, etc.) or unsaturations. As another alternative, R1 can be a hydrogen atom and R2 can be a vitamin D_3 fragment **K** as shown in Fig. 6B. This vitamin D_3 base also can include any appropriate substituent groups. R3, as shown in Fig. 5, can be any suitable substituent, such as a C_1 to C_7 alkyl group, straight chain, branched, aryl or formed into a ring. The R3 group can include any suitable substituent group, so long as the substituent group does not substantially compete or interfere with the desired reactions.

In accordance with the process of the invention, the unsaturated alkyl ketone starting material reacts with a chiral oxazaborolidine reagent to stereoselectively reduce the unsaturated

alkyl ketone to an unsaturated alcohol **D** or **E**. The selection of a particular oxazaborolidine reagent will determine the stereoselective orientation of the final product. If a chiral oxazaborolidine reagent having one stereospecific orientation is used, then the reaction mechanism shown at the top in Fig. 5 will be followed. If a chiral oxazaborolidine reagent is selected having the opposite stereospecific orientation, then the reaction mechanism shown at the bottom of Fig. 5 will be followed. Examples of suitable oxazaborolidine reagents include the borane complexes of (*S*)-MeCBS, (*S*)-BuCBS, (*R*)-MeCBS, or (*R*)-BuCBS (see Figs. 13A to 13D). These reagents are described in more detail below.

The intermediate unsaturated alcohol materials **D** and **E** are produced with a stereospecific orientation. The unsaturated bond in the alkyl chain can be further reduced in the process of the invention, if desired, to produce a saturated alkyl alcohol **F** or **G**, as shown in Fig. 5. If this saturated alcohol product is not the desired final product, it can be used as a convenient, stereospecific intermediate to produce the desired final product.

A more specific reaction scheme for obtaining material **F** in Fig. 5 now will be described. First, the starting material **C** must be produced, and in this reaction scheme, material **C** will be an alkeneone material. The alkeneone material **C** can be produced from an aldehyde substituent on the fused ring base. As shown in Fig. 7, the aldehyde substituent (material **A**) reacts with a Wittig reagent **B** to produce the alkeneone material **C**. R1, R2, and R3 in Fig. 7 have the same definitions as those described above in connection with Figs. 5, 6A, and 6B. The substituent X on the Wittig reagent in Fig. 7 can stand for a Ph_3P-group, an $(EtO)_2PO$-group, or an $(R4O)_2PO$-group, wherein R4 is an alkyl chain having 1-7 carbons, straight chain, branched, cyclic, or aryl.

The alkeneone material **C** reacts with the borane complex of (*R*)-MeCBS (**13**) and (*R*)-BuCBS (**14**) to stereoselectively reduce the alkeneone **C** to an allylic alcohol **D**. See the upper reaction mechanism in Fig. 5. (*R*)-MeCBS (**13**) and (*R*)-BuCBS (**14**) are borane complexes of oxazaborolidine reagents, the structures of which are illustrated in Figs. 13C and 13D, respectively. These reagents, without the borane complex (BH_3), are commercially available from Callery. The preparation of the borane complexes of the oxazaborolidine reagents is described in more detail below. Finally, as shown in Fig. 5, the allylic alcohol **D** is further reduced to eliminate the unsaturation bond and produce a hydroxylated, saturated alkane **F**.

An alternative mechanism for producing the saturated alkane **F** is described in conjunction with Figs. 8 and 9. In this instance, alkane **F** is produced through an alkyneone starting material **C**. The alkyneone starting material **C** first must be produced. As shown in Fig. 9, alkyneone material **C** also can be produced from an aldehyde starting material **A**. In a first reaction step, the aldehyde substituent on starting material **A** reacts to produce an alkyne alkyl substituent (material **H**) on the fused ring base. Thereafter, the alkyne **H** reacts with a lithium containing reagent and anhydride **I** to produce an alkyneone **C** on the fused ring base. In Fig. 9, R1, R2, and R3 have the same definitions as those described above.

Once produced, the alkyneone material **C** reacts with the borane complexes of (*S*)-MeCBS (**11**) or (*S*)-BuCBS (**12**) to stereoselectively reduce the alkyneone to a propargyllic alcohol **D**. See Fig. 8. (*S*)-MeCBS (**11**) and (*S*)-BuCBS (**12**) are borane complexes of oxazaborolidine reagents, the structure of which are illustrated in Figs. 13A and 13B, respectively. These reagents, without the borane complex, are commercially available from

Callery. The preparation of the borane complexes of the oxazaborolidine reagents is described in more detail below. Finally, the propargyllic alcohol **D** is further reduced to eliminate the unsaturation and produce a hydroxylated, saturated alkane **F**.

Once the hydroxyl group is provided at the C-24 position, as shown in Figs. 5 and 8, any suitable functional group can be attached to the steroid via the oxygen at the hydroxyl. For instance, an acetate group, a benzoate group, a TMS-O-group, or a phosphate group can be attached at the C-24 position in a stereospecific manner.

The process according to the invention now will be described in more specific terms in a process for producing specific steroid chemicals. These specific examples should be construed as illustrating the invention, and not as limiting the same.

I. Example - Production of Compounds Having a C-24 Alcohol Substituent (Compound 1436 and Squalamine)

A. Production of Test Materials and Standards

Squalamine is a steroid that contains a sulfated 24*R*-alcohol, as described in R. M. Moriarty, *et al.* "Stereoselective Synthesis of Squalamine Dessulfate," *Tetrahedron Letters*, **1995**, Vol. 36, No. 29, pp. 5139-5142. This article is entirely incorporated herein by reference. Squalamine is a member of a class of natural aminosterols from the shark that has clinical potential as an anti-infective (note K. S. Moore, *et al.* "Squalamine: An Aminosterol Antibiotic from the Shark," *Proc. Natl. Acad. Sci. USA*, **1993**, Vol. 90, 1354-1358) and an anti-angiogenic agent (note H. Brem, *et al.,* American Association of Neurological Surgeons, April 30 - May 4, 1996, Minneapolis, MN). The Moore article also is entirely incorporated herein by reference.

Squalamine contains a cholestane ring system with 5α-hydrido, 7α-hydroxyl, 3β-spermidinyl, and 24*R*-sulfate groups. Compound 1436 is similar in structure to squalamine, but it has a 3β-spermine substituent instead of the 3β-spermidinyl substituent.

Initially, it was desired to prepare a model system including compound **6**, as shown in Fig. 11A. This model system of compound **6** was desired because compound **6** contains many of the features required to synthesize squalamine and compound 1436, but it lacks the 7-hydroxyl group. Note the general correspondence between compound **6** and general compound **C** illustrated in Figs. 5, 7, 8, and 9. Compound **6** would be used to synthesize the 24*S*-allylic alcohol shown as compound **7**, which would then be reduced to produce the desired 24*R*-alcohol, shown as compound **9**. This general reaction scheme is illustrated in Fig. 11B. Note that the 22-23 double bond changes the priority of the groups in determining *R* from *S* in the nomenclature rules.

Fig. 12 generally illustrates the overall reaction process for producing compound **6**, and from compound **6**, producing compounds **9** and **10**, the standard samples. To avoid obscuring the process, details of each reaction step are not included in this portion of the application text. These reaction steps are described in more detail below in the "Experimental Section" of this application.

Initially, steroid **1** (commercially available from Pharmacia or Upjohn) was reduced with lithium in ammonia to produce material **2**. Notably, material **2** has the trans-AB-ring junction that is contained in squalamine. The carbonyl group at the C-3 position then was protected by

converting it to an ethylene ketal, thereby producing material 3. Material **3** was oxidized using pyridinium chlorochromate to produce the aldehyde material **4**.

In the next reaction step, material **4** was reacted with a Wittig reagent **5** to produce material **6**. This Wittig reagent **5** can be prepared in the manner described in the Experimental Section of this application. During this reaction, the cholest-22-ene-24-one side chain of substrate **6** was introduced in a single step from the C-22 aldehyde **4** via reaction with the Wittig reagent **5**. Material **6** was produced in good yield by this reaction procedure.

After material **6** was produced, this material was reduced to produce the alcohol products. Initially, two alcohol products **9** and **10** were prepared so that they could be compared by ^{13}C NMR spectroscopy. It is known that the NMR signal due to C-24 including an *R*-alcohol is 0.4 ppm upfield from that of the corresponding *S*-alcohol (with a saturated side chain). See N. Koizumi, *et al.*, "Carbon-13 Nuclear Magnetic Resonance of 24-Substituted Steroids," *Chem. Pharm. Bull.*, **1979**, Vol. 27, No. 1, pp. 38-42, which document is entirely incorporated herein by reference.

Alcohols **9** and **10** were produced as follows. Compound **6** was reduced with lithium aluminum hydride to produce a mixture of allylic alcohols **7** and **8**. The desired 24-*S* allylic alcohol (**7**) is produced if the hydride attacked material **6** from the alpha-face. The less polar compound (alcohol **8**) was separated and then reduced with hydrogen (Pd/C). A mixture of compounds **7** and **8** was also reduced for comparison, because pure compound **7** could not be separated from the mixture of compounds **7** and **8**. It was found that the less polar allylic alcohol **8** (faster moving on silica gel thin layer chromatography ("TLC") plates) yielded the undesired

24*S* alcohol **10**, as evidenced by a resonance at 77.66 ppm in the ^{13}C NMR spectrum. The mixture of **7** and **8** was reduced to produce saturated alcohols **9** and **10**, with resonances at 77.31 and 77.66. From these tests, applicants determined that the more polar allylic alcohol **7** must have the 24*S*-stereochemistry, and compound **9** must have the desired 24*R*-stereochemistry.

The reaction with compound **6** was similar to that described in a paper by Shen Zheng-Wu and Zhou Wei-Shan, "Study on the Syntheses of Brassinolide and Related Compounds. Part 14. Highly Stereoselective Construction of the Side-Chain of Brassinosteroids Utilizing the β-Alkylative 1,3-Carbonyl Transposition of the Steroidal 22-En-24-one," *J. Chem. Soc. Perkin Trans.*, 1990, Vol. 1, pp. 1765-1767. This article is entirely incorporated herein by reference. Zheng-Wu and Wei-Shan described that methyl lithium addition to a cholestane-22-ene-24-one system was achieved selectively from the alpha-face.

B. Selective Production of Compounds 7 and 9

1. Using Corey-Bakshi-Shibata Reagents ("CBS reagents")

In one method according to the invention, compound **6** is stereoselectively reduced to compound **7** using Corey-Bakshi-Shibata type reagents ("CBS reagents"). E.J. Corey described reducing non-steroidal α,β-unsaturated ketones with oxazaborolidine-borane complexes **11** and **12** (see Figs. 13A and 13B, respectively, for the chemical structure of complexes **11** and **12**) to selectively yield the *R*-allylic alcohols. See E.J. Corey, *et al.*, "A New System for Catalytic Enantioselective Reduction of Achiral Ketones to Chiral Alcohols. Synthesis of Chiral α-Hydroxy Acids," *Tetrahedron Letters*, **1990**, Vol. 31, No. 5, pp. 611-614 and U.S. Patent No.

4,943,635 dated July 24, 1990. These Corey documents each are entirely incorporated herein by reference. Corey further described the use of the opposite catalysts **13** and **14** (see Figs. 13C and 13D, respectively) to yield the *S*-allylic alcohols. See E.J. Corey, *et al.*, "Total Synthesis of (±)-Forskolin," *Journal of the American Chemical Society*, **1988**, Vol. 110, pp. 3672-3673. This document also is entirely incorporated herein by reference.

Suitable oxazaborolidines, such as (R)-MeCBS and (S)-MeCBS, are commercially available from Callery. These reagents are combined with borane to form the complexes shown in Figs. 13C and 13A. (R)-MeCBS is the compound of Fig. 13C without the BH_3 group. The production of the borane complex is described in more detail below, in the Experimental Section.

In view of the information of Corey, compound **6** was reduced with reagents **13** and **14** to produce the desired *S*-allylic alcohol **7**. A series of reductions were attempted on compound **6**, and the results are shown in the following Table.

Table. Reduction of Compound **6** with (*R*)-(X)-CBS at -20°C in Toluene

Entry	Catalyst (mol %)	BH_3-THF (eq.)	Addition time (hr)	Reaction time (hr)	de (%)*	Isolated yield (%)
1	Me-CBS (20)	1	1.5	21	40	
2	Bu-CBS (20)	2	1.5	3	0	
3	Me-CBS (20)	1+1+0.5	1.5	22		
4	Me-CBS (20)	2.5	2.3	22	30-35	

Entry	Catalyst (mol %)	BH_3-THF (eq.)	Addition time (hr)	Reaction time (hr)	de (%)*	Isolated yield (%)
5	Me-CBS (50)	2.5	2.3	2	80	56
6	Me-CBS (100)	1.5	2.5	22	40	
7	Me-CBS (100)	2.5	1.75	3	94-98	71

* diastereomeric excess, estimated by calibrated TLC on crude products

As demonstrated by this Table, a wide range of selectivities were produced in this reaction. The selectivities ranged from poor (entries 1-4, 6), to good (entry 5), to excellent (entry 7). The optimum conditions shown in the Table involved the use of stoichiometric quantities of (*R*)-Me-CBS with 2.5 equivalents of borane (from borated tetrahydrofuran ("BH_3-THF")), which provided a good yield (71%) and excellent selectivity (94-98% de by quantitative TLC) for compound **7**. The initial experiment was done with purification by column chromatography. Later experiments demonstrated that this reaction could be accomplished without chromatography in high yield (90%) to produce only compound **7** within the detection limits of ^{13}C NMR (>95%). Compound **7** was reduced with hydrogen to produce the saturated alcohol compound **9**, which had a carbon resonance of 77.29 ppm, confirming the earlier assignment (upfield approximately 0.4 ppm from compound **10**, located at 77.66 ppm).

2.Using (*S*)-methyl oxazaborolidine

*As an alternative method according to the invention, stereoselective reduction of the 22-yne-24-one system (see Fig. 8) with Corey's (S)-methyl oxazaborolidine reagent also was successful in introducing the chiral hydroxy group at the C-24 position. The substrate for the reduction, propargyl ketone **16**, was prepared from aldehyde **4** in two steps, in the manner shown in Figure 14. As noted above, the process steps will be set forth in more detail in the "Experimental Section" of this application.

In this alternative process, aldehyde **4** was homologated to the terminal alkyne **15** in a 97% yield with Seyferth's diazophosphonate reagent. See Seyferth, *et al.*, "Some Reactions of Dimethylphosphono-Substituted Diazoalkanes, $(MeO)_2P(0)$ CR Transfer to Olefins and 1,3-Dipolar Additions of $(MeO)_2P(0)$ $C(N_2)R^1$," *J. Org. Chem.*, **1971**, Vol. 36, pp. 1379-1386 (this article is entirely incorporated herein by reference). The reaction was carried out using the methodology developed by Colvin and Gilbert. See Colvin, *et al.*, "A Simple Procedure for the Elaboration of Carbonyl Compounds into Homologous Alkynes," *J. Chem Soc., Perkin Trans. I*, **1977**, pp. 869-874, and Gilbert, *et al.*, "Elaboration of Aldehydes and Ketones to Alkynes: Improved Methodology," *J. Org. Chem.*, **1979**, Vol. 44, No. 26, pp. 4997-4998, which articles each are entirely incorporated herein by reference. One portion of the terminal alkyne **15** was converted to the lithium alkynyltrifluoro borate and reacted with isobutyric anhydride to produce the propargyl ketone **16** (note the discussion in Brown et al., "Improved Highly Efficient Synthesis of α,β-Acetylenic Ketones. Nature of the Intermediate from the Reaction of Lithium Acetylide with Boron Trifluoride Etherate," *Tetrahedron Letters*, **1984**, Vol. 25, No. 23, pp. 2411-2414, which article is entirely incorporated herein by reference). The stereoselective

reduction of the propargyl ketone **16** was carried out using two equivalents of (*S*)-methyl CBS oxazaborolidine reagent and boron methyl sulfide ("BMS") in tetrahydrofuran at -30°C (see Parker, K.A. and Ledeboer, M.R., "Asymmetric Reduction. A Convenient Method for the Reduction of Alkynyl Ketones," *J. Org. Chem.*, **1996**, Vol. 61, pp. 3214-3217, which article is entirely incorporated herein by reference) to produce propargyl alcohol **17**. The propargyl alcohol **17** was hydrogenated to alcohol **9**, as shown in Fig. 14.

For comparison purposes, an epimeric mixture of propargyl alcohols **17** and **18** was prepared by treating a second portion of compound **15** with *n*-BuLi, followed by addition of isobutyraldehyde. The propargyl alcohols **17** and **18** were hydrogenated to give a mixture of alcohols **9** and **10**. ^{13}C NMR spectra of alcohols **9** and **10** produced two resonances of equal intensities at 77.29 and 77.63 ppm for the C-24 carbon (i.e., for the *R* and *S* stereochemistries, respectively).

The ^{13}C NMR spectra of the alcohol **9** (prepared via compounds **16** and **17** in Fig. 14) was compared to the ^{13}C NMR spectra of the epimeric mixture of alcohols **9** and **10**. The ^{13}C NMR spectra of the hydrogenated product of compound **17** showed a single signal for the C-24 carbon at 77.29 ppm corresponding to the 24 (*R*) stereochemistry. This confirmed that compound **9** was produced from compounds **4, 15, 16,** and **17** in a stereospecific manner.

C.Production of Steroids Including a 7α-Hydroxyl Group

1.Using Steroid 21 as a Starting Material

With these positive results in hand relating to the selective production of a 24(*R*)-hydroxyl group, applicants sought a process for the synthesis of compound **32** (see Fig. 15B), which can be used to produce a steroid having a 7α-hydroxyl group, as is present in squalamine and compound 1436. This intermediate (compound **32**) can be used in preparing squalamine and compound 1436. As mentioned above, a more detailed description of the production of the various compounds and intermediates is provided in the "Experimental Section" of this patent application.

The procedure for producing compound 1436 is illustrated in Figs. 15A and 15B. This specific description for compound 1436 also should be considered in conjunction with the more generalized process illustrated in Figs. 10A and 10B.

The procedure began with steroid **21**, which is a relatively inexpensive steroid, commercially available in bulk from Pharmacia or Upjohn. This steroid **21** was converted to the ketal **22** with migration of the olefin (see Fig. 15A). Olefin migration was necessary to direct oxidation at the C-7 position. Of course, as more generally shown in Fig. 10A, the specific ketal **22** of Fig. 15A is not necessary. Any suitable protecting groups located at the C-3 carbon can be used. For example, the R5 groups shown in Fig. 10A can be the same or different, and each can be an alkyl group having 1-6 carbon atoms. Furthermore, the two R5 groups can join together to form, for example, an ethylene dioxy group, a 1,3-propanedioxy group, a 2-methylene-1,3-propanedioxy group, or a 2,2-dimethyl-1,3-propanedioxy group.

Thereafter, the C-22 is protected as a *t*-butyldimethylsilyl ether (compound **23**) as shown in Fig. 15A. This procedure also is illustrated more generally in Fig. 10A. The protecting group

R6 at the C-22 position of compound **123**, as shown in Fig. 10A, need not be a *t*-butyldimethylsilyl ether compound. Rather, any suitable ether group can be used. In Fig. 10A, R6 can be, for example, formyl, acetyl, propionyl, pivaloyl, cyanoacetyl, benzoyl, substituted benzoyl (ortho or para substituted w/ nitro, halogen, alkoxy), methoxycarbonyl (methylcarbonate), ethoxycarbonyl, benzyloxycarbonyl, benzyl, substituted benzyl (*o,p*- nitro, *p*-halo, *p*-methoxy), benzyloxymethyl (BOM), substituted benzyloxymethyl (*o,p*- nitro, *p*-halo, *p*-methoxy), tetrahydrothiopyranyl, tetrahydrothiofuranyl, methylthiomethyl (MTM), trialkylsilyl (alkyl = methyl, ethyl, isopropyl, *sec*-butyl, *tert*-butyl, phenyl, or any combination of these), tetrahydropyranyl (THP), 2-methoxyethoxymethyl (MEM), and methoxymethyl (MOM).

Once the C-22 substituent of compound **23** is properly protected, allylic oxidation was achieved with chromium hexacarbonyl and *t*-butyl hydroperoxide, or by free radical air oxidation (see J. Foricher, *et al.*, U.S. Patent No. 5,030,739 dated July 9, 1991) to produce enone compound **24**. This U.S. patent is entirely incorporated herein by reference. This enone compound (compound **24**) was hydrogenated to compound **25**, and then treated with K-Selectride® (potassium tri-*sec*-butylborohydride, commercially available from Aldrich) to produce the 7α-alcohol **26**. In this portion of the synthesis method, the generalized process, shown in Fig. 10A, corresponds to the steps of the process shown in Fig. 15A.

The alcoholic hydroxyl group at C-7 (compound **26**) was protected as the benzoate (compound **27**). Instead of a benzoate group, any suitable protecting group R7 can be used at this point in the procedure, as illustrated generally in Fig. 10A. See compound **127**. The protecting group R7 at the C-7 position of compound **127** can be, for example, formyl, acetyl,

propionyl, pivaloyl, cyanoacetyl, benzoyl, substituted benzoyl (ortho or para substituted w/ nitro, halogen, alkoxy), methoxycarbonyl (methylcarbonate), ethoxycarbonyl, benzyloxycarbonyl, benzyl, substituted benzyl (*o,p*- nitro, *p*-halo, *p*-methoxy), benzyloxymethyl (BOM), substituted benzyloxymethyl (*o,p*- nitro, *p*-halo, *p*-methoxy), tetrahydrothiopyranyl, tetrahydrothiofuranyl, methylthiomethyl (MTM), trialkylsilyl (alkyl = methyl, ethyl, isopropyl, *sec*-butyl, *tert*-butyl, phenyl, or any combination of these), tetrahydropyranyl (THP), 2-methoxyethoxymethyl (MEM), and methoxymethyl (MOM).

Once the C-7 substituent group is properly protected, the C-22 alcohol, protected by the *t*-butyldimethylsilyl ether group, was liberated with fluoride anion to yield compound **28** (compound **128** in Fig. 10A). With the C-22 alcohol selectively available, oxidation under Swern conditions, or with bleach and TEMPO, cleanly produced the C-22 aldehyde **29** (compound **129** in Fig. 10A) in excellent yield, without epimerization of the potentially unstable group at C-20.

The aldehyde **29** (**129** in Fig. 10B) was reacted with either the ylide **5** or the anion of the phosphonate ester **30** to produce the enone **32** (**132** in Fig. 10B), which is required for the stereoselective reduction step. This is illustrated in Fig. 15B, and more generally in Fig. 10B. Reagent **30** is preferred in this reaction step because the by-products are easily removed upon work-up, making column chromatography unnecessary.

The chiral reduction conditions used in the model substrate **6** as described above worked well in this case to deliver the desired *S*-allylic alcohol **33** in high yield (compound **133** in Fig. 10B), without the need for chromatography. None of the other isomers was detected by TLC or

NMR spectroscopy. The proton NMR spectrum was compatible with the *S*-stereochemistry at the C-24 position in that the C-22-23 olefin signals were compressed (the olefin signals of the *R*-allylic alcohols tend to be more separated). Additionally, the carbon resonance for C-24 was at the identical position for compounds **7** and **33** (78.8 ppm), thereby indicating the presence of the hydroxyl at the C-24 position. After hydrogenation of compound **33**, an X-ray was performed on the hydrogenation product **34**, and this test confirmed the desired 24*R* assignment. The hydrogenation step is best performed in tetrahydrofuran or ethyl acetate (not in alcohol) in order to decrease the amount of deoxygenated products at C-24. The more generalized reaction scheme for these process steps is illustrated in Fig. 10B.

The final steps of the synthesis process, as shown in Figs. 10B and 15B, involved deprotection of the ketal at C-3 to produce compound **35** (**135** in Fig. 10B), sulfation of the C-24 alcohol (compounds **36** (Fig. 15B) and **136** (Fig. 10B)), and cleavage of the benzoate at C-7 (to produce compound **37**). Compounds **36**, **136**, and **37** are best isolated as their sodium or potassium salts. Finally reductive amination with spermine and sodium borohydride produced compound **38**, which corresponds to compound 1436 illustrated in Fig. 2. The overall yield of compound 1436 from the specific synthesis process shown in Figs. 15A and 15B was about 4%.

Squalamine (**42**) also can be produced by this same general process, although, when producing squalamine, compound **37** (Fig. 17) is coupled to the protected spermidine derivative **41** to afford the intermediate **43**. The nitrile functionality within **43** is reduced with hydrogen gas under platinum catalysis at acidic pH to afford squalamine (**42**) in approximately 60% yield for two steps. If this reduction were not performed at acidic pH, a very poor yield of squalamine

was obtained. In that case products were obtained from the internal nitrogen cyclizing on the nitrile.

Spermidine derivative **41** has been used as a spermidine equivalent in one other report (Umeda, et al., J. Antibiotics, 40, 1303-1315, 1987), but it has never been applied to the synthesis of squalamine. It is especially useful in this case because the protected amino function (the nitrile) is stable to reductive amination conditions, yet it is easily converted to an amino function (spermidine) by catalytic hydrogenation under conditions that do not affect the sulfate. The sulfate would be cleaved under strongly acidic conditions, as in the case of removal of a BOC-group. The spermidine derivative **41** was easily prepared in one step in good yield under the improved reaction conditions (73% versus 42% yield). The BOC-protected spermidine derivative used by Moriarty and Frye is more difficult to prepare and is not cleavable in the presence of a sulfate.

2.Using Steroid 50 as a Starting Material

The aldehyde **29** from Figs. 15A and 15B also can be prepared from the commercially available steroid stigmasterol, compound **50** shown in Figs. 16A and 16B, in seven steps. Fig. 16A shows a generalized reaction mechanism for producing compound **129**, and Fig. 16B shows a more specific example for producing the specific aldehyde **29**. The specific reaction steps of Fig. 16B also are described in more detail below, in the "Experimental Section" of this patent application.

In this process, first, stigmasterol **50** was oxidized in air, to give the unsaturated ketone **60**. The enone double bond was selectively reduced by dissolving metal reduction (Li/NH_3) to

produce the required α-hydrogen at the C-5 position (compound **61**). K-Selectride® (potassium tri-*sec*-butylborohydride from Aldrich) reduced the keto-functionality contained in compound **61** to yield an α-hydroxy group at the C-7 position (this is compound **62**). Selective oxidation of the C-3 hydroxyl group was achieved with silver carbonate to produce compound **63**. The hydroxyl group was then protected as the benzoate (compound **64**) in the process shown in Fig. 16B. As noted above in conjunction with Fig. 10A, however, protection as a benzoate is not necessary, but any appropriate protecting group R7 can be used. R7 in Fig. 16A has the same meaning as that described above in Fig. 10A.

Thereafter, the ketone at the C-3 position was protected as the ethylene ketal to produce compound **65** (Fig. 16B). Again, as described above in conjunction with Fig. 10A, any appropriate protecting group(s) R5 can be used in this process. R5 can have the same meaning in Fig. 16A as it has in Fig. 10A. Ozonolysis produced compound **29** (or compound **129** in Fig. 16A), which was identical to that prepared by the scheme shown in Fig. 15A.

This aldehyde **29** can be used to produce compound 1436 and/or squalamine, by the same process as that illustrated in Fig. 15B.

In the processes described above, the examples for R6 are not the only possible protecting groups for the C-22 position. Suitable protection R6 for the C-22 hydroxyl varies. The protecting group need only be removable under conditions where the C-3 and C-7 protecting groups are stable. Depending upon which point in the synthesis where the C-22 is protected, appropriate protection varies. If C-22 is protected prior to the allylic oxidation, protecting groups sensitive toward the oxidation conditions are not preferred (e.g., sulfur containing

protecting groups). Hydrogen labile protecting groups also are less suitable if applied prior to the hydrogenation; however, virtually any OH protecting group not requiring strongly acidic conditions for removal is acceptable if applied immediately prior to K-Selectride® reduction (K-Selectride® is potassium tri-*sec*-butylborohydride available from Aldrich). Readily cleaved esters are particularly useful in that they allow for selective cleavage in the presence of another more stable ester at C-7. Benzyl ethers, benzyloxymethyl ethers, and carbonates are most useful if applied after the first hydrogenation.

As with R6, the C-7 OH protection R7 should not require strongly acidic conditions for removal. Virtually all esters and silyl ethers are suitable for R7 as long as R6 can be removed in its presence.

An alternative procedure for forming compound 134 from compound 129 is illustrated in Fig. 10C. This procedure uses the alkyneone intermediates described above in conjunction with Figs. 8, 9 and 14. In Fig. 10C, R5 and R7 have the definitions described above with respect to Figs. 10A and 10B.

D. Conclusion Regarding Production of C-24 Hydroxylated Steroids

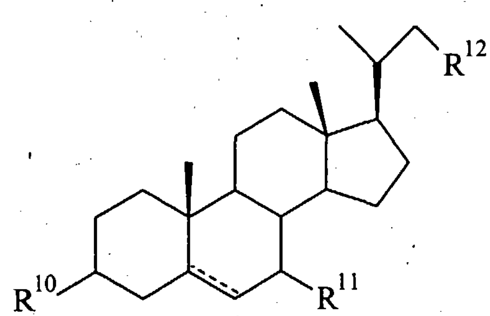
Suitable cholest-22-ene-24-one and cholest-22-yne-24-one systems can be easily constructed in one and two steps, respectively, from a C-22 aldehyde. These systems can then be selectively reduced with Corey's oxazaborolidine-borane complexes to produce the 24-*S*-allylic alcohol and 24-*S*-propargylic alcohols. These materials correspond to the 24-*R* alcohol after removal of the C-22-23 multiple bonds (i.e., the unsaturation bonds between the C-22 and C-23

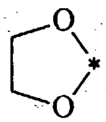
carbons). This procedure has been demonstrated on compounds **6**, **16**, and **32**. These two or three step procedures provide the most rapid access in a practical, scalable way to the aminosterol class of compounds that include a C-24*R* alcohol or other substituent. The C-24R alcohol can be converted to a sulfate grouping or other suitable substituent at the C-24 position. The process in accordance with the invention eliminates the lengthy procedures used by others to do the same transformation, as described in the documents noted above.

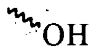
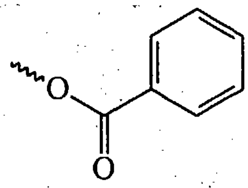
II. Intermediates

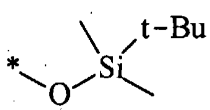
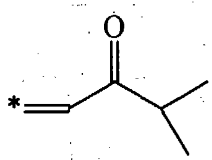
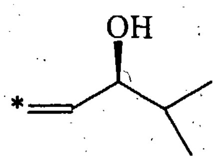
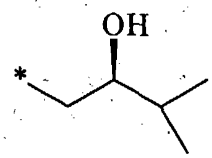
This invention also relates to the several intermediates that are identified in Figs. 10A, 10B, 10C, 12, 14, 15A, 15B, 16A and 16B. The intermediates in accordance with the invention are the compounds shown in the above-noted Figures, except for compounds 1, 2, 21, 22, 38, 50, 60, and 122. These compounds are intermediates useful in the production of various compounds including aminosterols such as squalamine and compound 1436. These aminosterols can be used in pharmaceutical products.

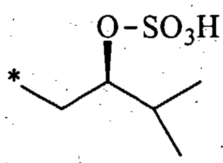
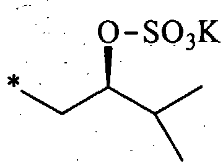
In one embodiment, this invention relates to intermediates formed in the synthesis of squalamine or homologous aminosterols. These intermediates have the composition:



wherein R^{10} is O═, , or 

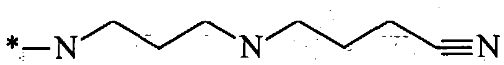
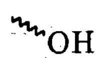
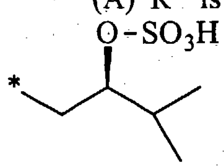
R^{11} is H, O═, , or ;

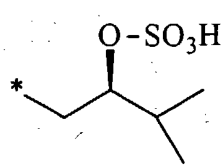
R^{12} is OH, , O═, , , ,

, or ;

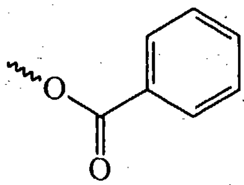
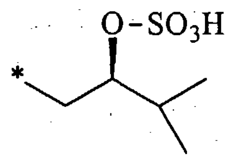
 represents either a single or double bond; and

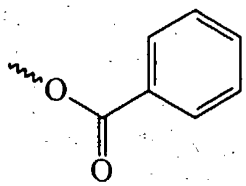
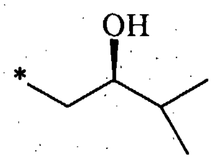
* represents the location where the R^{10} or R^{12} moieties are bonded to the remainder of the steroid molecule. Specific intermediates include the following compounds according to the above general formula, wherein:

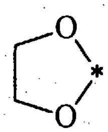
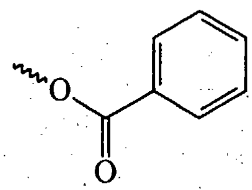
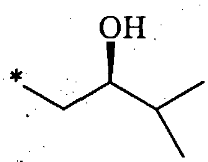
(A) R^{10} is , R^{11} is , R^{12} is , and  represents a single bond;

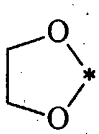
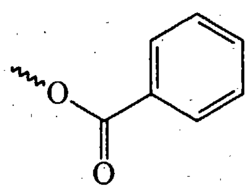
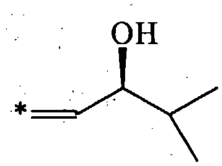
(B) R^{10} is O═, R^{11} is , R^{12} is , and  represents a

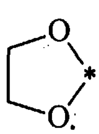
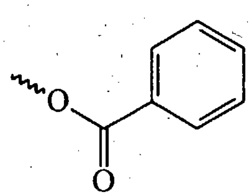
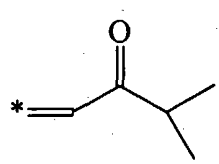
single bond;

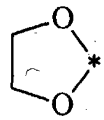
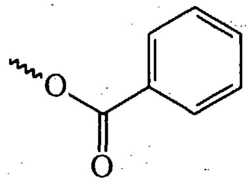
(C) R^{10} is $O=$, R^{11} is , R^{12} is , and  represents a single bond;

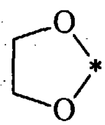
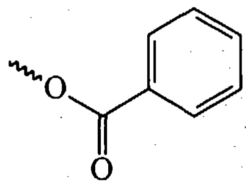
(D) R^{10} is $O=$, R^{11} is , R^{12} is , and  represents a single bond;

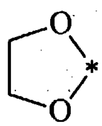
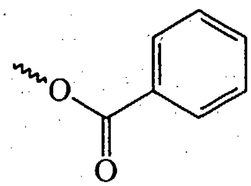
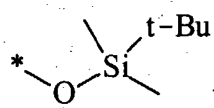
(E) R^{10} is , R^{11} is , R^{12} is , and  represents a single bond;

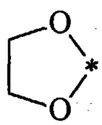
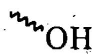
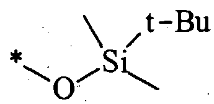
(F) R^{10} is , R^{11} is , R^{12} is , and  represents a single bond;

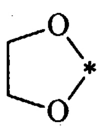
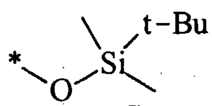
(G) R^{10} is , R^{11} is , R^{12} is , and  represents a single bond;

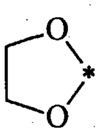
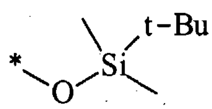
(H) R^{10} is , R^{11} is , R^{12} is O=, and  represents a single bond;

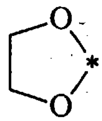
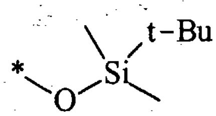
(I) R^{10} is , R^{11} is , R^{12} is OH, and  represents a single bond;

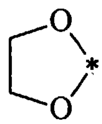
(J) R^{10} is , R^{11} is , R^{12} is , and  represents a single bond;

(K) R^{10} is , R^{11} is , R^{12} is , and  represents a single bond;

(L) R^{10} is , R^{11} is O=, R^{12} is , and  represents a single bond;

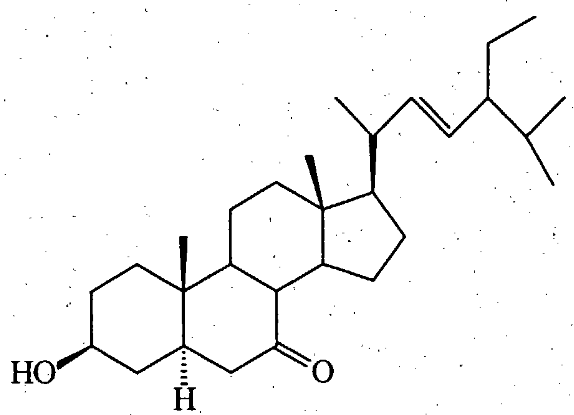
(M) R^{10} is , R^{11} is O=, R^{12} is , and  represents a double bond;

(N) R^{10} is  , R^{11} is H, R^{12} is  , and  represents a double bond; and

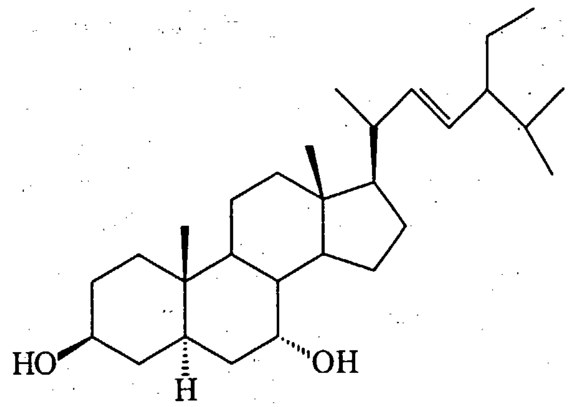
(O) R^{10} is  , R^{11} is H, R^{12} is OH, and  represents a double bond.

Particularly preferred intermediates include intermediates **61** through **65** shown in Fig. 16B. These intermediates are as follows:

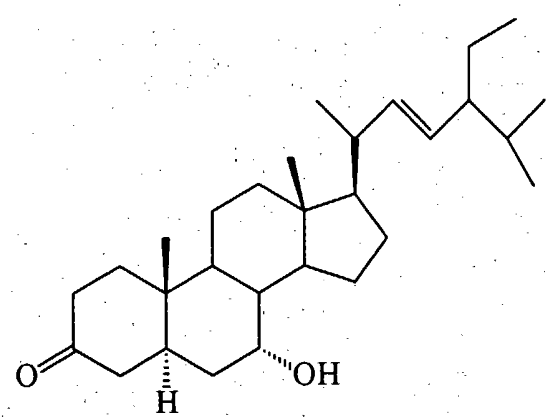
Compound **61**



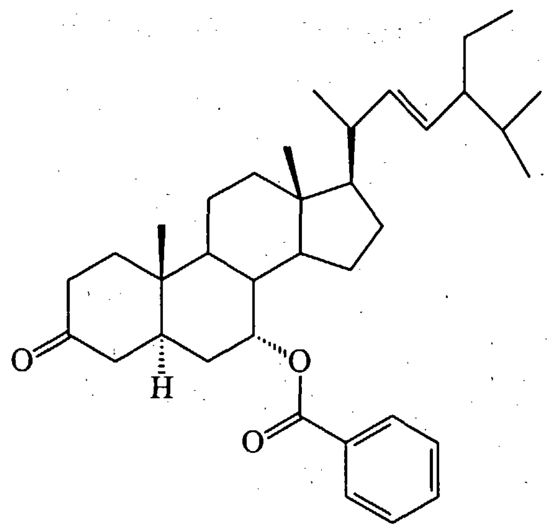
Compound **62**



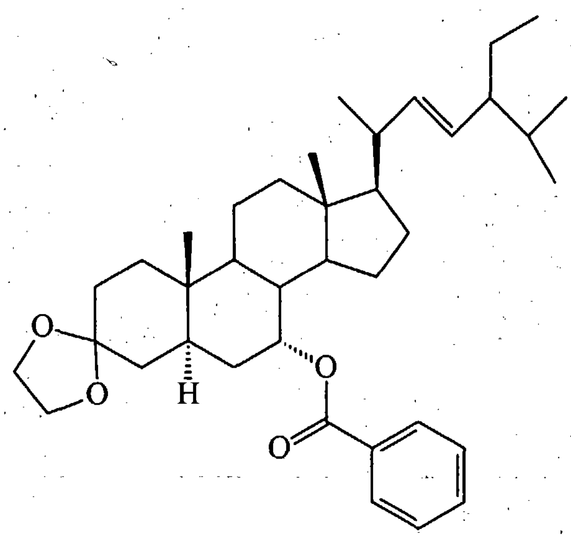
Compound **63**



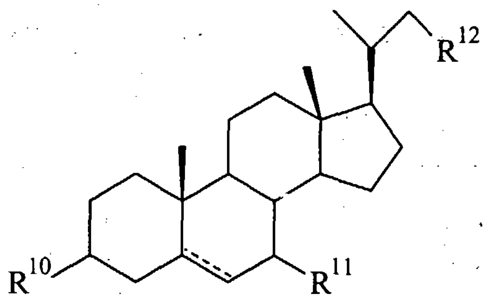
Compound **64**



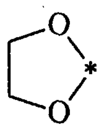
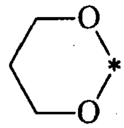
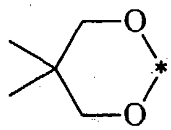
Compound **65**

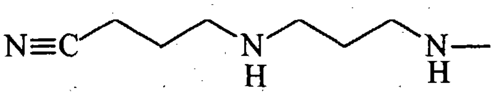
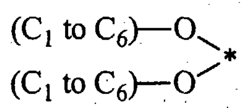


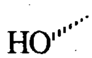
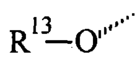
In another aspect, intermediates according to the invention can be defined by the following general formula:

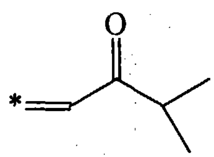
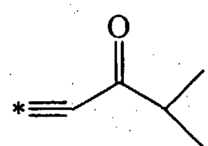
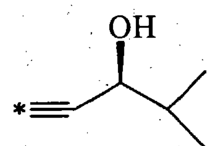


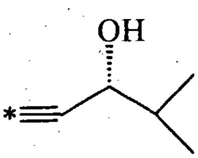
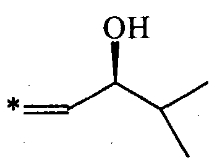
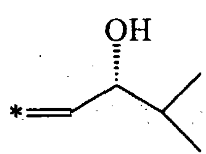
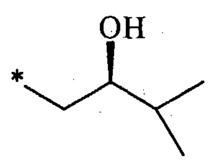
wherein:

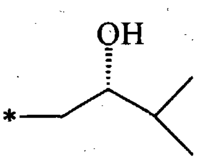
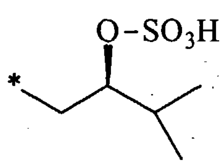
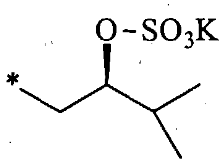
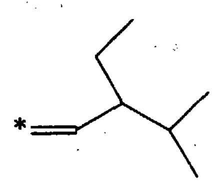
R^{10} is O=, , , ,

, , or ;

R^{11} is H, O=, , or ;

R^{12} is OH, —OR^{14}, O=, , , ,

, , , ,

, *≡C—H, , ; or 

 represents either a single or double bond;

* represents the location where the R^{10} or R^{12} moieties are bonded to the remainder of the steroid molecule;

C_1 to C_6, each independently represents an alkyl, alkenyl, or alkynyl group, having 1 to 6 carbon atoms, wherein the group may be substituted or unsubstituted; and

R^{13} and R^{14}, each independently represents formyl; acetyl; propionyl; pivaloyl; cyanoacetyl; benzoyl; benzoyl ortho or para substituted with nitro, halogen, or alkoxy; methoxycarbonyl (methylcarbonate); ethoxycarbonyl; benzyloxycarbonyl; benzyl; benzyl ortho or para substituted with nitro; benzyl para substituted with a halogen; benzyl para substituted with a methoxy; benzyloxymethyl; benzyloxymethyl ortho or para substituted with nitro; benzyloxymethyl para substituted with a halogen; benzyloxymethyl para substituted with a methoxy; tetrahydrothiopyranyl; tetrahydrothiofuranyl; methylthiomethyl; trialkylsilyl, wherein each alkyl is independently selected from the group of methyl, ethyl, isopropyl, *sec*-butyl, *tert*-butyl, and phenyl; tetrahydropyranyl; 2-methoxyethoxymethyl; and methoxymethyl.

III. Experimental Section

A. General

Unless otherwise noted, 77.23 ppm was used as the reference for $CDCl_3$ in ^{13}C NMR experiments.

B.Preparation of Specific Compounds

1.Preparation of Compound **2** (Fig. 12)

Ammonia (60 ml) was condensed into a flask under nitrogen, and lithium wire (98 mg, 14 mmol) was added. Steroid **1** (Fig. 12) (1.0 g, 3.0 mmol) was dissolved in anhydrous

tetrahydrofuran (25 ml) and added dropwise. Steroid **1** is commercially available from Pharmacia or Upjohn. After 40 minutes, the reaction was quenched with solid ammonium chloride until the blue color disappeared, and then the mixture was allowed to evaporate overnight. The resulting solid was partitioned between water (150 ml) and ethyl acetate (200 ml). The aqueous layer was extracted with portions of ether and dichloromethane, and the combined organic layers were washed with brine, dried over sodium sulfate, and evaporated to yield a white solid. This material was dissolved in dichloromethane and purified by flash chromatography (gradient elution with 10 to 40% ethyl acetate in hexane) to produce compound **2** (710 mg, 71%, mp 168-170°C).

Compound **2**: ^{1}H NMR (400 MHZ, $CDCl_3$): δ 3.64 (d of d, J=10 and 2 Hz, 1H), 3.36 (d of d, J=10 and 3 Hz, 1H), 2.33-1.08 (m, 24H), 1.05 (d, J = 6.7 Hz, 3H), 1.02 (s, 3H), 0.71 (s, 3H); ^{13}C NMR (400 MHZ, $CDCl_3$): δ 212.4, 68.1, 56.2, 53.9, 52.7, 46.8, 44.9, 42.8, 39.9, 38.9, 38.7, 38.4, 35.8, 35.6, 31.9, 29.1, 27.9, 24.5, 21.6, 16.9, 12.3, 11.6; Anal. Calcd. for $C_{22}H_{36}O_2$: C, 79.46; H, 10.91. Found: C, 79.54; H, 10.48.

2.Preparation of Compound **3**

A solution of compound **2** (710 mg, 2.14 mmol), ethylene glycol (1.13 ml, 20 mmol), and *p*-toluenesulfonic acid monohydrate (41 mg, 0.21 mmol) in benzene (90 ml) was heated at reflux overnight with the removal of water by a Dean-Stark trap. The reaction mixture was cooled, washed with saturated sodium bicarbonate solution and brine, dried over sodium sulfate, and evaporated to yield compound **3** (725 mg, 90%, mp 184-186°C).

Compound **3**: ^1H NMR (400 MHZ, $CDCl_3$): δ 3.94 (s, 4H), 3.64 (d of d, J = 10.3 and 3.1 Hz, 1H), 3.36 (d of d, J = 10.3 and 6.8 Hz, 1H), 1.98 - 1.94 (m, 1H), 1.83 - 1.76 (m, 1H), 1.7 - 1.0 (m, 22H), 1.04 (d, J = 7.0 Hz, 3H), 0.82 (s, 3H), 0.68 (s, 3H); ^{13}C NMR (400 MHZ, $CDCl_3$): δ 109.6, 68.2, 64.3, 56.4, 54.2, 52.7, 43.9, 42.9, 40.1, 39.0, 38.2, 36.2, 35.9, 35.7, 35.6, 32.1, 31.4, 28.8, 27.9, 24.5, 21.4, 16.9, 12.4, 11.6; IR (KBr, cm^{-1}): 3315, 2930, 1447, 1360, 1179, 1101; Anal. Calcd. for $C_{24}H_{40}O_3$: C, 76.55; H, 10.71. Found: C, 74.91; H, 10.06.

3.Preparation of Compound **4**

A suspension of potassium acetate (140 mg, 1.43 mmol) and pyridinium chlorochromate (1.09 g, 5.06 mmol) in dichloromethane (20 ml) was treated with compound **3** (1.0 g, 2.65 mmol) in dichloromethane (10 ml). After 1.25 hours, the reaction mixture was diluted with ether and filtered through Celite® (Celite® is a form of SiO_2 sold by Aldrich). The ether layers were combined, washed with saturated sodium bicarbonate solution and brine, dried over sodium sulfate, and evaporated to give a white solid. This material was purified by flash chromatography (gradient elution with 5 to 25% ethyl acetate in hexane) to provide compound **4** (604 mg, 61%, mp 141-144°C).

Compound **4**: ^1H NMR (400 MHZ, $CDCl_3$): δ 9.56 (d, J = 3.5 Hz, 1H), 3.94 (s, 4H), 2.34 (m, 1H), 1.93 - 0.86 (m, 23H), 1.11 (d, J = 6.5 Hz, 3H), 0.82 (s, 3H), 0.70 (s, 3H); ^{13}C NMR (400 MHZ, $CDCl_3$): δ 205.4, 109.6, 64.3, 55.9, 54.2, 51.3, 49.7, 43.8, 43.4, 39.9, 38.1, 36.2, 35.7, 32.1, 31.4, 28,7, 27.2, 24.8, 21.3, 13.6, 12.7, 11.6; IR (KBr, cm^{-1}): 3478, 2934, 2725, 1721, 1445, 1356, 1101; MS (+FAB): 375.3 (M+1, 60), 307.1 (100), 289.1 (45).

4.Preparation of Compound **6**

A solution of compound **4** (820 mg, 2.19 mmol) and compound **5** (1.52 g, 4.38 mmol) in methyl sulfoxide (4 ml) was heated to 110°C overnight, cooled, dissolved in ethyl acetate, washed with water, and dried. The crude material was purified by flash chromatography (gradient elution with 5 to 15% ethyl acetate in hexane) to yield compound **6** (720 mg, 74%, mp 168-169°C). A procedure for preparing compound **5** and its reaction with C-22 aldehydes are described in M. Fryberg, A.C. Oehlschlager, and A.M. Unrau, "The Synthesis of Possible Polyene Intermediates in Phytosterol Biosynthesis," *Tetrahedron*, **1971**, Vol. 27, pp. 1261-1274. This article is entirely incorporated herein by reference.

Compound **6**: ^{1}H NMR (400 MHZ, $CDCl_3$): δ 6.71 (d of d, J = 16 and 9 Hz, 1H), 6.06 (d, J = 16 Hz, 1H), 3.94 (s, 4H), 2.83 (hept, J = 7 Hz, 1H), 2.25 (m, 1H), 1.95 (m, 1H), 1.7-1.0 (m, 22H), 1.11 - 1.08 (m, 9H), 0.82 (s, 3H), 0.69 (s, 3H); ^{13}C NMR (400 MHZ, $CDCl_3$): δ 204.9, 152.9, 126.3, 109.6, 64.3, 56.5, 55.2, 54.2, 43.8, 43.2, 40.2, 40.0, 38.4, 38.1, 36.2, 35.6, 32.0, 31.3, 28.7, 28.4, 24.4, 21.3, 19.5, 18.8, 18.7, 12.6, 11.6; Anal. Calcd. for $C_{29}H_{46}O_3$-0.1H_2O: C, 78.36; H, 10.48. Found: C, 78.21; H, 10.68.

5.Preparation of Compounds **7** and **8**

A solution of compound **6** (100 mg, 0.226 mmol) was dissolved in anhydrous tetrahydrofuran (2 ml), treated with 1 M lithium aluminum hydride in tetrahydrofuran (380 μl, 0.38 mmol), and refluxed for 1 hour under nitrogen. After cooling, the reaction was quenched with methanol, filtered through Celite® (SiO_2, available from Aldrich), and purified by flash chromatography (gradient elution with 7 to 10% ethyl acetate in hexane) to provide pure compound **8** (18 mg, 18%, mp 147-150°C, less polar by TLC 5% acetone/chloroform), followed

by two mixed fractions of compound **7** (more polar) and compound **8** (49 + 15 mg = 64 mg, 64%).

Compound **8**: [1]H NMR (400 MHZ, $CDCl_3$): δ 5.49 (d of d, J = 15.4 and 8.2 Hz, 1H), 5.37 (d of d, J = 15.4 and 7.0 Hz, 1H), 3.94 (s, 4H), 3.77 (t, J = 6.4 Hz, 1H), 2.3-1.0 (m, 25H), 1.02 (d, J = 6.5 Hz, 3H), 0.92 (d, J = 6.7 Hz, 3H), 0.87 (d, J = 6.8 Hz, 3H), 0.82 (s, 3H), 0.68 (s, 3H); [13]C NMR (400 MHZ, $CDCl_3$): δ 139.4, 128.6, 109.6, 78.42 (C24-*R*), 64.3, 56.6, 56.0, 54.2, 43.9, 42.8, 40.1, 40.0, 38.1, 36.2, 35.6, 34.1, 32.1, 31.3, 28.8, 24.4, 21.4, 20.6, 18.4, 12.5, 11.7, 11.6; Anal. Calcd. for $C_{29}H_{48}O_3$: C, 78.33; H, 10.88. Found: C, 78.24; H, 10.87.

Mixture of compounds **7** and **8**: [13]C NMR (400 MHZ, $CDCl_3$): δ 78.80, 78.37 (C24-*S* and *R*).

6.Synthesis of Compound **10**

A solution of compound **8** (75 mg, 0.17 mmol) in ethyl acetate (4 ml) was treated with 10% palladium on carbon (76 mg) and hydrogen (40 psi) on a Parr apparatus for 6 hours. The reaction mixture was filtered through Celite® (SiO_2, available from Aldrich), evaporated, and recrystallized (ethyl acetate in hexane) to provide compound **10** (18 mg, 24 %, mp 122-132°C).

Compound **10**: [1]H NMR (400 MHZ, $CDCl_3$): δ 3.30 (m, 1H), 2.0 - 1.0 (m, 29H), 0.93 - 0.89 (m, 9H), 0.81 (s, 3H), 0.65 (s, 3H); [13]C NMR (400 MHZ, $CDCl_3$): δ 109.7, 77.64 (DEPT, C24-*S*), 64.4, 56.7, 56.2, 56.1, 54.2, 43.9, 42.8, 40.2, 38.2, 36.2, 36.1, 35.7, 35.6, 33.4, 32.4, 32.1, 31.4, 30.9, 30.0, 28.8, 28.4, 24.4, 21.4, 19.3, 19.0, 18.7, 18.6, 18.5, 16.9, 12.3, 11.6; MS (+FAB): 447.3 (M+1, 100), 90.9 (80); Anal. Calcd. for $C_{29}H_{50}O_3$: C, 77.97; H, 11.28. Found: C, 77.34; H, 10.84.

7.Synthesis of Compounds **9** and **10**

A mixture of compounds **7** and **8** (8.5 mg, 0.019 mmol) in ethanol (5 ml) was treated with 10% palladium on carbon (22 mg) and hydrogen (40 psi). The reaction mixture was filtered through Celite® (SiO_2, available from Aldrich) and evaporated to yield a mixture of compounds **9** and **10** (8 mg).

Mixture of compounds **9** and **10**: ^{13}C NMR (400 MHZ, DEPT, $CDCl_3$): δ 77.66, 77.31 (C24-*S* and *R*).

8.Stereoselective Synthesis of Compound **7**

(R)-diphenylprolinol (0.286 g, 1.13 mmol) and trimethylboroxane (0.14 g, 1.13 mmol) were combined in toluene (30 ml). This mixture was stirred at 50°C for 1 hour and then heated to reflux until 20 ml of an azeotropic mixture was distilled. After cooling, 1 M borane-tetrahydrofuran complex (2.8 ml, 2.8 mmol) was added at room temperature, and the solution was stirred for 2 hours. Then, a solution of compound **6** (0.50 g, 1.13 mmol) in toluene (15 ml) was added at -20°C over 1.75 hours. After an additional hour, the reaction was quenched with water (20 ml) and 5% hydrochloric acid (20 ml). After stirring for 30 minutes at room temperature, toluene (50 ml) was added, and the organic phase was washed with brine (3 x 20 ml) to pH 7. The organic phase was dried over magnesium sulfate, filtered, and concentrated *in vacuo* to yield compound **7** (0.48 g, de estimated by TLC ($CHCl_3$/*i*-Pr_2O 80:20) calibration = 94 - 98%). After purification by chromatography, the alcohol was obtained as a white solid (0.36 g, 72%, mp 157°C).

Compound **7**: ^{1}H NMR (400 MHZ, $CDCl_3$): δ 5.43 (d of d, J = 15.3 and 8.3 Hz, 1H), 5.34 (d of d, J = 15.3 and 7 Hz, 1H), 3.93 (s, 4H), 3.72 (t, J = 7 Hz, 1H), 2.05 (m, 1H), 1.93 (m, 1H), 1.8 - 1.0 (m, 23H), 1.02 (d, J = 6.6 Hz, 3H), 0.92 (d, J = 6.6 Hz, 3H), 0.86 (d, J = 6.5 Hz, 3H), 0.80 (s, 3H), 0.67 (s, 3H); ^{13}C NMR (400 MHZ, $CDCl_3$): δ 140.0, 128.6, 109.6, 78.82 (C24-*S*), 64.3, 56.7, 55.8, 54.2, 43.8, 42.8, 40.3, 40.1, 38.1, 36.2, 35.6, 34.1, 32.1, 31.3, 29.0, 28.7, 24.4, 21.4, 20.6, 18.6, 18.4, 12.4, 11.6; MS (+FAB): 445.3 (M+1, 48), 427.3 (37), 90.9 (100); Anal. Calcd. for $C_{29}H_{48}O_3$: C, 78.33; H, 10.88. Found: C, 78.22; H, 10.59.

9.Synthesis of Compound **9**

A solution of compound **7** (19 mg, 0.043 mmol) in ethyl acetate (10 ml) was treated with 10% palladium on carbon (5 mg) and 40 psi of hydrogen for 4 hours. The reaction was filtered, concentrated *in vacuo*, recrystallized from ethyl acetate in hexane, and then purified by flash chromatography (1 cm diameter, gradient elution with 7 to 8% ethyl acetate in hexane) to provide compound **9** (11 mg, 57%, mp 125-127°C).

Compound **9**: ^{1}H NMR (400 MHZ, $CDCl_3$): δ 3.94 (s, 4H), 3.31 (m, 1H), 2.0-1.0 (m, 29H), 0.92 - 0.89 (m, 9H), 0.81 (s, 3H), 0.66 (s, 3H); ^{13}C NMR (400 MHZ, $CDCl_3$): δ 109.7, 77.29 (DEPT, C24-*R*), 64.3, 56.7, 56.3, 54.2, 43.9, 42.8, 40.2, 38.2, 36.2, 35.9, 35.7, 33.7, 32.2, 32.1, 31.4, 30.8, 28.8, 28.5, 24.4, 21.4, 19.1, 18.8, 17.4, 12.3, 11.6; MS (+FAB): 447.4 (M+1).

10.Preparation of Compound **15** (Fig. 14)

A solution of dimethyl diazomethylphosphonate (205 mg, 1.4 mmol) in THF (1 ml) was added dropwise to a solution of potassium *t*-butoxide (1.4 ml, 1M K^+ $^-$OtBu in THF, 1.4 mmol) in THF (2.4 ml) at -78°C. The resulting yellow solution was stirred for ten minutes. The aldehyde **4** (394 mg, 1.05 mmol) was dissolved in THF (5 ml) and cooled to -78°C. The cooled aldehyde solution was quickly transferred to the flask containing the phosphonate using a short cannula. The flask that contained aldehyde was rinsed with THF (3 ml), cooled, and then added in the same manner. The reaction was stirred at -78°C for about 12 hours and was allowed to warm to room temperature overnight. The reaction was quenched with saturated sodium bicarbonate solution and extracted with ether (4 x 25 ml). The ether layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give a crude solid. Silica gel chromatography using 15% ethyl acetate in hexanes gave the pure alkyne **15** as a white solid (380 mg, 97%, m.p. 173-175°C).

Compound **15**: ^{1}H NMR (400 MHZ., $CDCl_3$): δ 3.94 (s, 4H), 2.45 (m, 1H-20), 2.02 (d, J = 2 Hz., 1H-23), 1.21 (d, J = 7 Hz., 3H-21), 0.81 (s, 3H-19), 0.68 (s, 3H-18).
^{13}C NMR (100 MHZ., $CDCl_3$): δ 109.63, 89.68, 68.58, 64.35, 56.36, 55.59, 54.30, 43.90, 42.81, 39.46, 38.16, 36.23, 35.69, 35.61, 32.07, 31.36, 31.18, 28.74, 27.74, 27.45, 24.35, 21.51, 21.24, 12.61.

IR (KBr, cm^{-1}): 3257, 2104, 1255, 686.

MS (CI, isobutane): m/e (relative intensity): 371 ($[M+H]^+$, 100), 307 (20), 154 (82), 136 (72).

Anal. Calcd for $C_{25}H_{38}O_2$: C, 81.03; H, 10.34. Found: C, 80.85, H, 9.87.

11.Preparation of Compound **16**

A solution of *n*-BuLi in hexanes (0.5 ml, 1.6 M, 0.81 mmol) was added dropwise to a solution of alkyne **15** (100 mg, 0.27 mmol) in THF (5 ml) at -78°C. The reaction was stirred for one hour, and boron trifluoride diethyl etherate (0.1 ml, 0.81 mmol) was added dropwise. After stirring for 15 minutes at -78°C, isobutyric anhydride (0.2 ml, 1.2 mmol) was added in one portion. The reaction was stirred at -78°C for about 30 minutes and was quenched by adding 0.2 N NaOH solution. The reaction mixture was extracted with ether (3 x 10 ml), and the organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give a crude oil. Silica gel chromatography using 5% ethyl acetate in hexanes gave the pure propargyl ketone **16** as a thick oil (86 mg, 72%).

Compound **16**: ^{1}H NMR (400 MHZ., $CDCl_3$): δ 3.94 (s, 4H), 2.63 (m, 2H, H-20 & H-25), 1.95 (m, 1H), 1.83 (m, 1H), 1.26 (d, J = 6.8 Hz., 3H-21), 1.18 (d, J = 6.8 Hz., 6H, H-25 & H-26), 0.82 (s, 3H-19), 0.70 (s, 3H-18).

^{13}C NMR (100 MHZ., $CDCl_3$): δ 192.85, 109.59, 99.86, 80.62, 64.36, 56.16, 55.26, 54.28, 43.88, 43.33, 42.99, 39.33, 38.14, 36.23, 35.69, 35.62, 32.05, 31.35, 28.69, 28.18, 27.34, 24.39, 21.21, 20.64, 18.29, 12.78, 11.62.

IR (KBr, cm^{-1}): 2206, 1675.

MS (CI, isobutane): m/e (relative intensity): 441 ($[M+H]^+$, 85), 125 (23), 99 (100), 77 (35).

Anal. Calcd for $C_{29}H_{44}O_3$: C, 79.04; H, 10.06; Found: C, 78.45, H, 9.57.

12.Preparation of Compound **17**

A solution of propargyl ketone **16** (50 mg, 0.11 mmol) in THF (0.5 ml) was dried over 4 A molecular sieves for two hours. The ketone solution was then added via a syringe to a solution of (S)-MeCBS (0.18 ml, 1.3 M in toluene, 0.23 mmol) in THF (0.5 ml) at room temperature. The resulting solution was cooled to -30°C, and a solution of boron methyl sulfide in THF (0.28 ml, 2M in THF, 0.57 mmol) was added dropwise over 5-10 minutes. The reaction was stirred at -30°C for about an hour, at which time, the TLC indicated that the reaction was complete. The reaction was quenched by slowly adding methanol (1 ml). The solution was diluted with ether, washed with saturated ammonium chloride solution, followed by 5% sodium bicarbonate and then brine. The ether layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Silica gel chromatography using 20% ethyl acetate in hexanes gave the pure propargyl alcohol **17** as a white solid (36 mg, 72%).

Compound 17: ^1H NMR (400 MHZ., $CDCl_3$): δ 4.15 (m, 1H-24), 3.94 (s, 4H), 2.48 (m, 1H-20), 1.19 (d, J = 6.9 Hz., 3H-21), 0.98 (d, J = 6.7 Hz., 3H) 0.96 (d, J = 6.7 Hz., 3H), 0.81 (s, 3H-19), 0.67 (s, 3H-18).

^{13}C NMR (100 MHZ., $CDCl_3$): δ 109.38, 90.84, 80.16, 68.13, 64.09, 56.12, 55.68, 54.11, 43.67, 42.57, 39.22, 37.93, 36.00, 35.47, 35.39, 34.68, 31.85, 31.13, 28.52, 27.62, 27.32, 24.15, 21.41, 21.00, 18.16, 17.32, 12.50, 11.38. (NOTE: 77.00 ppm was used as reference).

IR (KBr, cm^{-1}): 3464, 2230.

13.Preparation of Compound **9**

A solution of propargyl alcohol **17** (35 mg, 0.08 mmol) in ethyl acetate (3 ml) was treated with 10% palladium on carbon (20 mg, 0.02 mmol), sodium nitrite (~2-3 mg) and hydrogen (40 psig) on a Parr apparatus for 17 hours. The reaction was filtered through a pad of Celite® (SiO_2, available from Aldrich), and the filtrate was concentrated *in vacuo*. The crude solid was purified by silica gel column chromatography using 20% ethyl acetate in hexanes to provide the alcohol **9** (27 mg, 77%).

Compound **9**: ^{1}H NMR (250 MHZ., $CDCl_3$): δ 3.92 (s, 4H), 3.32 (m, 1H-24), 1.95 (m, 1H), 1.83 (m, 1H), 0.90 (d, J = 6.8 Hz., 6H, H-25 & H-26), 0.80 (s, 3H-19), 0.65 (s, 3H-18). ^{13}C NMR (400 MHz, DEPT, $CDCl_3$): δ 77.29 (C24-R).

14.Preparation of Epimeric Mixture of Compounds **17** and **18**

A solution of *n*-BuLi in hexanes (0.2 ml, 1.6 M, 0.32 mmol) was added dropwise to a solution of alkyne **15** (40 mg, 0.11 mmol) in THF (2 ml) at -78°C. The reaction was stirred for 15 minutes and HMPT (0.2 ml, 1.1 mmol) was added. After stirring for another hour at -78°C, isobutyraldehyde (0.03 ml, 0.38 mmol) was added in one portion. The reaction was allowed to warm up to room temperature and was quenched by adding saturated ammonium chloride solution. The reaction mixture was extracted with ether (3 x 10 ml), and the organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give a crude oil. Silica gel column chromatography using 10% ethyl acetate in hexanes separated the unreacted starting material **15** from the product **17** and **18** (20 mg, 43%, m.p. 138 - 140°C).

Mixture of compounds **17** and **18**: ^{1}H NMR (400 MHZ., $CDCl_3$): δ 4.15 (m, 1H-24), 3.94 (s, 4H), 2.48 (m, 1H-20), 1.19 (d, J = 6.6 Hz., 3H-21), 0.98 (d, J = 7.9 Hz., 3H) 0.96 (d, J = 7.3 Hz., 3H), 0.81 (s, 3H-19), 0.68 (s, 3H-18).

^{13}C NMR (100 MHZ., $CDCl_3$): δ 109.63, 91.16, 80.28, 68.36, 64.35, 56.34, 55.87, 54.31, 43.90, 42.78, 39.44, 38.16, 36.23, 35.69, 35.61, 34.91, 32.08, 31.36, 28.74, 27.88,27.59, 24.39, 21.65, 21.24, 18.41, 17.55, 12.75, 11.63.

IR (KBr, cm^{-1}): 3464, 2229.

MS (CI, isobutane): m/e (relative intensity): 443 ($[M+H]^+$, 90), 154 (17), 125 (22), 99 (100).

Anal. Calcd for $C_{29}H_{46}O_3$: C, 78.68; H, 10.47; Found: C, 78.13, H, 10.02.

15.Preparation of Epimeric Mixture of Compounds **9** and **10**

A solution of a mixture of propargyl alcohols **17** and **18** (20 mg, 0.045 mmol) in ethyl acetate (3 ml) was treated with 10% palladium on carbon (10 mg, 0.02 mmol), sodium nitrite (~1 mg) and hydrogen (40 psig) on a Parr apparatus for 4 hours. The reaction was filtered through a pad of Celite® (SiO_2, available from Aldrich), and the filtrate was concentrated *in vacuo*. The crude solid was purified by silica gel column chromatography using 20% ethyl acetate in hexanes to produce the mixture of alcohols **9** and **10** (16 mg, 80%).

Mixture of compounds **9** and **10**: ^{1}H NMR (250 MHZ., $CDCl_3$): δ 3.92 (s, 4H), 3.32 (m, 1H-24), 1.95 (m, 1H), 1.83 (m, 1H), 0.90 (d, J = 6.8 Hz., 6H, H-25 & H-26), 0.80 (s, 3H-19), 0.65 (s, 3H-18). ^{13}C NMR (400 MHz, DEPT, $CDCl_3$): δ 77.63, 77.29 (C24-S+R).

In the procedures noted above, note the discussion in the Seyferth, Colvin, Gilbert, Brown, and Parker articles. Each of these articles is discussed above in this patent application.

16.Alternate Method for Synthesis of Compound **22** (Fig. 15A)

A solution of compound **21** (19 g, 57 mmol, commercially available from Pharmacia or Upjohn), ethylene glycol (180 ml, 3.2 mol), and *p*-toluenesulfonic acid monohydrate (2.72 g, 14.3 mmol) in toluene (700 ml) was heated to reflux with removal of water for 25 hours. After cooling, saturated sodium bicarbonate (200 ml) was added, and the organic phase was washed with brine (2 x 100 ml) and dried over sodium sulfate. After removing solvent, the product was purified by flash chromatography (gradient elution with 0 to 40% ethyl acetate in hexane) and recrystallized (ethyl acetate in hexane - 150 ml) to give compound **22** (15.98 g, 75 %, mp 178-180°C): ^{1}H NMR (400 MHZ, $CDCl_3$): δ 5.36 (m, 1H), 3.95 (m, 4H), 3.64 (d of d, J = 10.5 and 3.2 Hz, 1H), 3.37 (d of d, J = 10.5 and 7.0 Hz, 1H), 2.59 - 2.55 (m, 1H), 2.12 (d of d, J = 14.1 and 3.0 Hz, 1H), 2.0-1.0 (m, 19H), 1.06 (d, J = 6.8 Hz, 3H), 1.04 (s, 3H), 0.71 (s, 3H); ^{13}C NMR (400 MHZ, $CDCl_3$): δ 140.3, 122.3, 109.6, 68.1, 64.6, 64.4, 56.6, 52.5, 49.8, 42.6, 41.9, 39.7, 38.9, 36.7, 36.5, 32.0, 31.8, 31.2, 27.9, 24.5, 21.2, 19.0, 16.9, 12.1; Anal. Calcd. for $C_{24}H_{38}O_3$: C, 76.96; H, 10.23. Found: C, 76.88; H, 9.67.

16b.Conditions for Synthesis of Compound **22**

Chlorotrimethylsilane (3.0 ml, 24 mmol) was added to a mixture of compound **21** (1.78 g, 5.4 mmol) and ethylene glycol (25 ml). After 27 hours at room temp, the reaction mixture was treated with 5% sodium bicarbonate solution (50 ml), extracted with ethyl acetate (3 x 70 ml), washed with brine (5 x 15 ml), water (15 ml), and dried over magnesium sulfate. After removing

the solvent, the product was recrystallized from ethanol (25 ml) to give compound **22** (1.41 g, 2 crops, 70%, mp 180 - 182 °C).

17. Synthesis of Compound **23**

A mixture of compound **22** (4.2 g, 11.2 mmol), imidazole (1.53 g, 22.47 mmol), 4-dimethylaminopyridine (192 mg, 1.57 mmol), and *tert*-butyldimethylsilyl chloride (2.61 g, 17.3 mmol) in anhydrous dimethylformamide (60 ml) was stirred for 4 hours at room temperature, and then diluted with ether (150 ml) and methylene chloride (50 ml), washed with water (3 x 50 ml), and dried over sodium sulfate. After removing the solvent, the product was crystallized from ethyl acetate and hexane (100 ml) to yield pure compound **23** (5.41 g, 98 %, mp 131-133°C): ^{1}H NMR (400 MHZ, $CDCl_3$): δ 5.35 (m, 1H), 3.95 (m, 4H), 3.59 (d of d, J = 9.6 and 3.2 Hz, 1H), 3.23 (br t, J = 9 Hz, 1H), 2.59 - 2.55 (m, 1H), 2.12 (d of d, J = 14.2 and 2.5, 1H), 2.1 - 1.0 (m, 19H), 1.03 (s, 3H), 0.99 (d, J = 6.2 Hz, 3H), 0.89 (s, 9H), 0.69 (s, 3H), 0.03 (s, 6H); Anal. Calcd. for $C_{30}H_{52}O_3Si$: C, 73.71; H, 10.72. Found: C, 73.28; H, 10.10.

18. Synthesis of Compound **24**

A solution of compound **23** (3.42 g, 7.0 mmol), chromium hexacarbonyl (420 mg, 1.9 mmol), and 90% *tert*-butyl hydroperoxide (2.2 ml, 19.8 mmol) in acetonitrile (100 ml) was heated to reflux for 20 hours. After cooling, the mixture was diluted with ether (150 ml), washed with brine (3 x 30 ml), and dried over sodium sulfate. After evaporation, the product was purified by flash chromatography (gradient elution with 0 to 30% ethyl acetate in hexane) to produce starting material (compound **23**, 510 mg) and compound **24** (1.53 g, 51% based on recovered starting material, mp 147 - 149°C): ^{1}H NMR (400 MHZ, $CDCl_3$): δ 5.63 (s, 1H), 3.94 (m, 4H), 3.57 (m, 1H), 3.22 (t, J = 8.4 Hz, 1H), 2.39 (br d, J = 7 Hz, 1H), 2.4 - 1.0 (m, 18H), 1.18 (s, 3H), 0.97 (d, J = 6.2 Hz, 3H), 0.86 (s, 9H), 0.67 (s, 3H), 0.00 (s, 6H); ^{13}C NMR (400 MHZ, $CDCl_3$): δ 201.9, 164.6, 126.8, 109.0, 68.0, 64.7, 64.6, 51.4, 49.9, 49.7, 45.5, 43.4, 41.9, 39.1, 38.7, 38.6, 38.4, 35.8, 31.2, 28.2, 26.6, 26.1, 21.3, 18.5, 17.2, 17.1, 12.2, -5.2; MS (+FAB): 503.3 (M+1).

19. Alternative Synthesis of Compound **24**

A mixture of compound **23** (489 mg, 1 mmol), N-hydroxyphthalimide (326 mg, 2 mmol), and benzoyl peroxide (10 mg, 0.008 mmol) in *n*-butyl acetate (50 ml) was heated at 110°C and treated with air for 6 hours. The solvent was evaporated, and the residue was treated with dichloromethane (50 ml) and filtered. After washing the recovered N-hydroxyphthalimide (266 mg) with dichloromethane, the filtrate was evaporated, and the residue was dissolved in 5 ml of pyridine at 50°C, cooled to 5-10°C, treated with 0.5 ml (5.3 mmol) of acetic anhydride, and left at room temperature overnight. The solvent was evaporated, and the residue dissolved in 25%

ethyl acetate and purified by flash chromatography (2.5 cm diameter, gradient elution with 0 to 25% ethyl acetate in hexane) to produce pure compound **24** (398 mg, 77%).

20.Synthesis of Compound **25**

Hydrogenation of a solution of compound **24** (360 mg, 0.715 mmol) in ethyl acetate (25 ml) and ethanol (15 ml) was performed with 10% palladium on carbon (360 mg) at 40 psi of hydrogen on a Parr shaker. After 5 hours, the reaction mixture was filtered through Celite® (SiO_2, available from Aldrich), evaporated, and concentrated under vacuum overnight to yield compound **25** (350 mg, 97%), which was homogenous by TLC: ^1H NMR (400 MHZ, $CDCl_3$): δ 3.88 (m, 4H), 3.54 (d of d, J = 9 and 3 Hz, 1H), 3.22 (t, J = 9 Hz, 1H), 2.3 - 1.0 (m, 22H), 1.05 (s, 3H), 0.95 (d, J = 6.6 Hz, 3H), 0.85 (s, 9H), 0.63 (s, 3H), 0.01 (s, 6H); ^{13}C NMR (400 MHZ, $CDCl_3$): δ 211.8, 108.8, 67.9, 64.4, 64.3, 55.0, 51.6, 50.1, 48.7, 45.9, 45.7, 42.7, 39.0, 38.7, 37.9, 36.0, 35.2, 31.2, 28.0, 26.1, 25.2, 21.9, 18.5, 17.1, 12.3, 11.1, -5.2; MS (+FAB): 505.3 (M+1); Anal. Calcd. for $C_{30}H_{52}O_4Si$: C, 71.38; H, 10.38. Found: C, 71.57; H, 10.16.

21.Synthesis of Compound **26**

1 M K-Selectride® (potassium tri-*sec*-butylborohydride from Aldrich) in THF (8.5 ml, 8.5 mmol) was added dropwise to a solution of compound **25** (800 mg, 1.58 mmol) in tetrahydrofuran (35 ml) at -50°C. The reaction mixture was stirred for 5 hours and then quenched by the careful addition of 30% hydrogen peroxide (10 ml) and saturated sodium bicarbonate (20 ml). The aqueous layer was extracted with ether (3 x 50 ml), and the combined organic layers were washed with saturated sodium bicarbonate (2 x 20 ml), water (20 ml), and brine (20 ml). After drying, the solvent was removed, and the product was purified by flash

chromatography (gradient elution with 0 to 30 % ethyl acetate in hexane) to produce compound **26** (645 mg, 80%, mp 174-175°C): ^{1}H NMR (400 MHZ, $CDCl_3$): δ 3.93 (s, 4H) 3.82 (br s, 1H), 3.58 (br d, J = 8 Hz, 1H), 3.25 (br t, J = 8 Hz, 1H), 2.0-1.0 (m, 22H), 0.99 (d, J = 6.3 Hz, 3H), 0.89 (s, 9H), 0.82 (s, 3H), 0.67 (s, 3H), 0.03 (s, 6H); ^{13}C NMR (400 MHZ, $CDCl_3$): δ 109.4, 68.1, 68.0, 64.4, 52.8, 50.5, 45.8, 42.9, 39.7, 39.5, 39.3, 37.7, 36.5, 36.4, 35.9, 35.8, 31.4, 27.9, 26.2, 24.0, 21.1, 18.6, 17.1, 12.1, 10.6, -5.2.

22.Synthesis of Compound **27**

A solution of compound **26** (11.88 g, 23.4 mmol) in anhydrous pyridine (110 ml) under nitrogen was treated with 4-dimethylaminopyridine (3.43 g, 28.1 mmol) and benzoyl chloride (5.5 ml, 47.4 mmol) at room temperature, and then heated at reflux for 22 hours. The cooled reaction mixture was poured into saturated sodium bicarbonate (500 ml) and extracted with ethyl acetate (200 ml). The aqueous phase was extracted with more ethyl acetate (3 x 100 ml), and the combined organic layers were washed with saturated sodium bicarbonate (3 x 50 ml) and brine (2 x 50 ml), dried over sodium sulfate, and then concentrated to give crude product. The solid was recrystallized from ethyl acetate in methanol to obtain pure compound **27** (13.8 g, 96%, mp 180-183°C): ^{1}H NMR (400 MHZ, $CDCl_3$): δ 8.07 (d, J = 7 Hz, 2H), 7.59 (t, J = 7 Hz, 1H), 7.48 (t, J = 7 Hz, 2H), 5.15 (br s, 1H), 3.88 (m, 4H), 3.55 (d of d, J = 9.5 and 3.4 Hz, 1H), 3.15 (t, J = 9.5 Hz, 1H), 2.1 - 1.0 (m, 22H), 0.98 (d, J = 6.8 Hz, 3H), 0.88 (s, 3H), 0.86 (s, 9H), 0.69 (s, 3H), 0.01 (s, 6H); ^{13}C NMR (400 MHZ, $CDCl_3$): δ 166.2, 133.0, 131.2, 129.8, 128.6, 109.2, 72.2, 68.0, 64.4, 52.9, 50.7, 47.4, 43.1, 39.6, 39.3, 38.8, 37.5, 37.4, 35.9, 35.7, 33.5, 31.4, 27.7, 26.2,

24.0, 21.3, 18.6, 17.0, 12.1, 10.7, -5.2; Anal. Calcd. for $C_{37}H_{58}O_5Si$-$0.2H_2O$: C, 72.31; H, 9.58. Found: C, 72.13; H, 9.26.

23. Synthesis of Compound **28**

Tetrabutylammonium fluoride (1 M, 27 ml, 27 mmol) in tetrahydrofuran was added to a solution of compound **27** (10.8 g , 17.7 mmol) in anhydrous tetrahydrofuran (90 ml) at room temperature, and then the reaction was heated to reflux for 6.5 hours. After cooling, the mixture was diluted with ethyl acetate (200 ml) and washed with water (60 ml), brine (3 x 60 ml), and dried over sodium sulfate. After removing solvent, the product was purified by flash chromatography (gradient elution with 0 to 25% ethyl acetate in hexane) to yield compound **28** (8.14 g, 93%, mp 117-119°C): ^1H NMR (400 MHZ, $CDCl_3$): δ 8.06 (d, J = 8 Hz, 2H), 7.59 (t, J = 7 Hz, 1H), 7.48 (t, J = 8 Hz, 2H), 5.16 (br s, 1H), 3.88 (m, 4H), 3.58 (d of d, J = 10.5 and 3.3 Hz, 1H), 3.33 (d of d, J = 11 and 6.5 Hz, 1H), 2.1 - 1.1 (m, 22H), 1.04 (d, J = 6.9 Hz, 3H), 0.89 (s, 3H), 0.70 (s, 3H); ^{13}C NMR (400 MHZ, $CDCl_3$): δ 166.2, 133.0, 131.1, 129.8, 128.6, 109.2, 72.0, 68.0, 64.4, 64.3, 52.4, 50.7, 47.4, 43.0, 39.5, 38.8, 37.5, 37.4, 35.9, 35.6, 33.4, 31.4, 27.7, 23.9, 21.3, 16.9, 14.4, 12.0, 10.7; $C_{31}H_{44}O_5$-$0.2H_2O$: C, 74.42; H, 8.95. Found: C, 74.47; H, 8.79.

24. Alternate Method for Synthesis of Compound **29**

A solution of dimethylsulfoxide (3.0 ml, 42 mmol) in dichloromethane (15 ml) was added to a cold (-70 to -60°C) 2 M solution of oxalyl chloride in dichloromethane (9 ml, 18 mmol) under nitrogen. After 10 minutes, a solution of compound **28** (6.0 g, 12.1 mmol) in dichloromethane (55 ml) was added dropwise. After stirring for 1 hour, triethylamine (9 ml, 64

mmol) was added, and the reaction was allowed to warm to room temperature. Water (120 ml) was added, and the aqueous phase was extracted with dichloromethane (3 x 100 ml), which was in turn washed with brine (3 x 100 ml) and water (90 ml), and dried over sodium sulfate. After evaporation, clean product **29** was obtained, without further purification (6.0 g, 100%, mp 166-168°C): ^{1}H NMR (400 MHZ, $CDCl_3$): δ 9.52 (d, J = 2.4 Hz, 1H), 8.06 (d, J = 7 Hz, 2H), 7.60 (t, J = 7 Hz, 1H), 7.49 (t, J = 7 Hz, 2H), 5.17 (m, 1H), 3.88 (m, 4H), 2.35 (m, 1H), 2.0 - 1.1 (m, 21H), 1.11 (d, J = 6.9 Hz, 3H), 0.89 (s, 3H), 0.73 (s, 3H); ^{13}C NMR (400 MHZ, $CDCl_3$): δ 205.1, 166.1, 133.0, 131.0, 129.8, 128.7, 109.1, 71.9, 64.4, 64.3, 51.1, 50.2, 49.6, 47.4, 43.5, 39.4, 38.8, 37.5, 37.4, 35.9, 35.7, 33.4, 31.4, 27.1, 24.2, 21.2, 13.6, 12.3, 10.7.

24b.Conditions for Synthesis of Compound **29**

A solution of potassium bromide (1.44 g, 12.0 mmol) in water (60 ml) was added to a solution of alcohol **28** (60.0 g, 0.12 mol) and 2,2,6,6-tetramethyl-1-piperidine oxide (TEMPO) (360 mg, 2.4 mmol) in dichloromethane (600 ml). The mixture was cooled in an ice-water bath and stirred vigorously. A sodium hypochlorite solution was prepared by diluting commercial Clorox bleach (5.28%) with an equal volume of water to become 2.64% (0.39 M, 324 ml, 0.12 mol, pH 11.3). This Clorox solution was adjusted to pH 9.5 by adding sodium bicarbonate powder while keeping the temperature between 10 - 15 °C. The sodium hypochlorite solution was added dropwise to the mixture above, and the reaction mixture was stirred for 30 minutes. The aqueous layer was separated and extracted with dichloromethane (2 x 100 ml). The combined dichloromethane extracts were washed with water (2 x 150 ml) and brine (50 ml), dried over magnesium sulfate, and evaporated. The residue (69.1 g) was triturated in refluxing

hexane (200 ml) for 30 minutes and cooled to room temperature and then in the refrigerator for 1 hour. The white powder was collected by filtration, washed with hexane (2 x 40 ml) and dried (50 °C, 0.1 mm, 5 hours) to give pure aldehyde **29** (47.5 g, 79%, mp 164-166 °C). If full strength bleach is used, the reaction fails in that aldehyde **29** is obtained as a mixture of C-20 isomers.

25.Preparation of Compound **30** (Fig. 15B)

A solution of 1-bromo-3-methyl-2-butanone (Note M. Gaudry and A. Marquet, "1-Bromo-3-Methyl-2-Butanone," *Organic Synthesis*, Vol VI, pp. 193-195, which article is entirely incorporated herein by reference) (10 g, 60 mmol) and triethyl phosphite (10.3 ml, 60 mmol) was heated to 120°C under nitrogen for 3 hours with the removal of ethyl bromide by distillation. The cooled reaction mixture was placed under high vacuum and then distilled to produce compound **30** (8.0 g, 60%, bp 127-130°C, 3 mm) (procedure from the thesis of Phu H. Le, UCSD, **1983**, T.C. McMorris): ^{1}H NMR (400 MHZ, $CDCl_3$): δ 4.15 (p, J = 7 Hz, 4H), 3.14 (d, J = 22.5 Hz, 2H), 2.87 (heptet, 7 Hz, 1H), 1.34 (t, J = 7 Hz, 6H), 1.13 (d, J = 7 Hz, 6H).

26.Preparation of Compound **32**

Sodium hydride (60%, 44 mg, 1.1 mmol) was washed with heptane (2 ml) and hexane (2 x 2 ml), and evaporated with a nitrogen flow. Anhydrous tetrahydrofuran (2 ml), compound **30** (0.34 ml, 1.5 mmol), and a solution of compound **29** (486 mg, 0.982 mmol) in tetrahydrofuran (3 ml) were added. The reaction mixture was heated to reflux for 1 hour, cooled to room temperature, and treated with water (25 ml). The aqueous layer was extracted with ethyl acetate (3 x 50 ml), which was in turn washed with brine (3 x 50 ml), water (2 x 50 ml), and dried over sodium sulfate. After removing solvent, the product was dissolved in methanol (5 ml)

containing 4 drops of pyridine, and dropped into water (100 ml) with shaking. The resulting solid was filtered, washed with water (3 x 20 ml), and dried under vacuum at 50°C to give pure compound **32** (499 mg, 90%, mp 85 - 121°C): ^{1}H NMR (400 MHZ, $CDCl_3$): δ 8.06 (d, J = 7.4 Hz, 2H), 7.59 (t, J = 7.1 Hz, 1H), 7.48 (t, J = 7.4 Hz, 2H), 6.65 (d of d, J = 15.6 and 9 Hz, 1H), 6.02 (d, J = 15.6 Hz, 1H), 5.15 (br s, 1H), 3.88 (br s, 4H), 2.80 (hept, J = 6.7 Hz, 1H), 2.25 (m, 1H), 2.0-1.0 (m, 21H), 1.08 (d, 3H), 1.06 (d, J = 6.9 Hz, 6H), 0.89 (s, 3H), 0.72 (s, 3H); ^{13}C NMR (400 MHZ, $CDCl_3$): δ 204.7, 166.1, 152.5, 133.0, 131.1, 129.8, 128.6, 126.5, 109.2, 72.0, 64.4, 64.3, 55.0, 50.8, 47.4, 43.2, 40.1, 39.5, 38.8, 38.3, 37.5, 37.4, 35.9, 35.7, 33.4, 31.4, 28.2, 23.8, 21.2, 19.5, 18.8, 18.6, 13.6, 12.2, 10.7; MS (+FAB): 563.3 (M+1); Anal. Calcd. for $C_{36}H_{50}O_5$-0.2H_2O: C, 76.34; H, 8.97. Found: C, 76.26; H, 9.13.

In this process, the procedure of T.C. McMorris, et al., "Synthesis of Dehydro-Oogoniol, a Female-Activating Hormone of ACHLYA: The Progesterone Route," *Steroids* ,**1989**, Vol. 53, pp. 345-361 was followed. This article is entirely incorporated herein by reference.

27.Preparation of Compound **33**

An argon blanketed flask was charged with 1 M *R*-MeCBS (from Callery) in toluene (0.92 ml, 0.92 mmol) and 1 M borane-tetrahydrofuran complex in tetrahydrofuran (2.3 ml, 2.3 mmol). The reaction mixture was stirred at room temperature for 2 hours, cooled to -20°C, and treated with a solution of compound **32** (520 mg, 0.92 mmol) in anhydrous toluene (15 ml) over 1.5 hours. After an additional hour, the reaction mixture was treated with solid ammonium chloride and water (2 ml), warmed to room temperature, diluted with more water (20 ml), and extracted into toluene (2 x 80 ml). The toluene layer was washed with saturated ammonium

chloride (3 x 50 ml), dried with magnesium sulfate, and evaporated to give a solid, which was recrystallized from ethyl acetate in hexane to give compound **33** in two crops (491 mg, 94%, mp 196-199°C): ^{1}H NMR (400 MHZ, $CDCl_3$): δ 8.06 (d, J = 7 Hz, 2H), 7.59 (t, J = 7 Hz, 1H), 7.48 (t, J = 7 Hz, 2H), 5.35 (m, 2H), 5.16 (br s, 1H), 3.88 (br s, 4H), 3.67 (t, J = 6.2, 1H), 2.1-1.0 (m, 23H), 1.03 (d, J = 6.3 Hz, 3H), 0.89 (m, 6H), 0.82 (d, J = 6.6 Hz, 3H), 0.70 (s, 3H); ^{13}C NMR (400 MHZ, $CDCl_3$): δ 166.1, 139.8, 133.0, 131.2, 129.9, 128.8, 128.6, 109.2, 78.8, 72.0, 64.4, 64.3, 55.6, 51.0, 47.4, 42.9, 40.2, 39.6, 38.8, 37.5, 37.4, 35.9, 35.7, 34.1, 33.5, 31.5, 28.8, 23.9, 21.3, 20.6, 18.6, 18.4, 12.2, 10.7; MS (+FAB): 565.3 (M+1); Anal. Calcd. for $C_{36}H_{52}O_5$-0.2H_2O: C, 76.07; H, 9.29. Found: C, 75.93; H, 9.14.

28. Alternate Method for Synthesis of Compound **34**

A solution of compound **33** (2.7 g, 4.8 mmol) in tetrahydrofuran (30 ml) was treated with sodium nitrite (89 mg, 1.3 mmol), 20% palladium hydroxide on carbon (0.5 g, Pearlman's catalyst), and 40 psi of hydrogen on a Parr apparatus. After 16 hours, the reaction mixture was filtered through Celite® (SiO_2, available from Aldrich) and concentrated to obtain crude material, which was recrystallized from dichloromethane in hexane (15 ml) to produce pure compound **34** (2.13 g, 78%, mp 205-208°C): ^{1}H NMR (400 MHZ, $CDCl_3$): δ 8.08 (d, J = 7.5 Hz, 2H), 7.60 (t, J = 7.5 Hz, 1H), 7.49 (t, J = 7.5 Hz, 2H), 5.16 (br s, 1H), 3.88 (m, 4H), 3.28 (m, 1H), 2.0 - 1.0 (m, 27H), 0.93 - 0.90 (m, 9H), 0.89 (s, 3H), 0.69 (s, 3H); ^{13}C NMR (400 MHZ, $CDCl_3$): δ 166.2, 133.0, 131.2, 129.9, 128.6, 109.2,77.0, 72.1, 64.4, 64.3, 56.0, 50.8, 47.3, 42.9, 39.6, 38.7, 37.5, 37.3, 35.8, 35.6, 33.6, 33.4, 32.1, 31.4, 30.7, 28.2, 23.8, 21.2, 19.1, 18.8, 17.4,

11.9, 10.7; MS (+FAB): 567.5 (M+1); Anal. Calcd. for $C_{36}H_{54}O_5$-0.3H_2O: C, 75.56; H, 9.62. Found: C, 75.29; H, 9.04.

28b.Conditions for Synthesis of Compound **34**

A mixture of compound **33** (100 mg, 0.18 mmol), 10% platinum on carbon (5 mg), triethylamine (5 drops) in ethyl acetate (15 ml) was treated with a hydrogen balloon for 22 hours. After filtration through Celite®, the solution was evaporated and recrystallized from methanol (1 ml) and water (few drops) to afford approx. 95% pure compound **34** (81 mg, 80%).

29.Preparation of Compound **35**

A solution of compound **34** (210 mg, 0.37 mmol) in 90% acetone/water (16 ml) was treated with pyridinium *p*-toluenesulfonate (73 mg, 0.29 mmol) and heated to reflux for 12 hours. The reaction mixture was extracted with ethyl acetate (25 ml), and this material subsequently was washed with water (2 x 25 ml), dried over sodium sulfate, and evaporated to yield compound **35** (170 mg, 88%): ^1H NMR (400 MHZ, $CDCl_3$): δ 8.03 (d, J = 7 Hz, 2H), 7.60 (t, J = 7 Hz, 1H), 7.48 (t, J = 7 Hz, 2H), 5.20 (br s, 1H), 3.28 (m, 1H), 2.5 - 1.0 (m, 27H), 1.09 (s, 3H), 0.93 (d, J = 6.3 Hz, 3H), 0.90 (d, J = 7 Hz, 3H), 0.89 (d, J = 7 Hz, 3H), 0.72 (s, 3H); ^{13}C NMR (400 MHZ, $CDCl_3$): δ 211.5, 165.9, 133.2, 130.9, 129.8, 128.7, 71.5, 56.1, 50.8, 47.2, 44.1, 42.9, 40.6, 39.6, 38.8, 38.4, 38.3, 35.8, 33.7, 32.1, 30.7, 28.3, 23.8, 21.6, 19.1, 18.8, 17.4, 12.0, 10.9; Anal. Calcd. for $C_{34}H_{50}O_4$-0.3H_2O: C, 77.32; H, 9.66; Found: C, 77.06; H, 9.23.

30.Alternative Method for Synthesis of Compound **36**

Sulfur trioxide-pyridine complex (610 mg, 3.8 mmol) and compound **35** (830 mg, 1.59 mmol) were dissolved in anhydrous pyridine (7 ml) under nitrogen. The reaction mixture was

heated to 80°C for 3 hours, evaporated, dissolved in dichloromethane (400 ml), washed with water (100 ml) and brine (2 x 100 ml), dried, and evaporated to obtain compound **36** (1.06 g, 98%) as the pyridine salt: ^{1}H NMR (400 MHZ, $CDCl_3$): δ 8.91 (m, 2H), 8.36 (t, J = 7 Hz, 1H), 7.99 (d, J = 7 Hz, 2H), 7.91 (m, 2H), 7.57 (t, J = 7 Hz, 1H), 7.46 (t, J = 7 Hz, 2H), 5.15 (br s, 1H), 4.26 (m, 1H), 2.4 - 1.0 (m, 27H), 1.06 (s, 3H), 0.88 (m, 9H), 0.66 (s, 3H); ^{13}C NMR (400 MHZ, $CDCl_3$): δ 211.5, 165.9, 145.4, 142.7, 133.2, 130.7, 129.6, 128.7, 127.2, 85.1, 71.4, 56.0, 50.7, 47.1, 44.0, 42.8, 40.5, 39.4, 38.6, 38.3, 38.2, 35.7, 33.6, 31.1, 30.9, 28.0, 26.8, 23.7, 21.4, 18.7, 18.3, 17.8, 11.9, 10.8; MS (-LD): 606.

30b.Conditions for Synthesis of Compound **36**

A mixture of compound **34** (17.6 g, 31 mmol), acetone (880 ml), and Amberlyst® 15 ion-exchange resin (7.1 g, Aldrich 21,638-0) was stirred at room temperature for 3 hours. At this time HPLC showed 3.5% unconverted compound **34**. After filtration and washing with acetone (3 x 25 ml), pyridine (2 ml) was added to the filtrate. After evaporation of the solvent *in vacuo*, pyridine (100 ml) was added. Evaporation *in vacuo* was continued until another 75 ml of distillate was obtained. Pyridine (500 ml) was added to the residue, and this solution was used without further purification. The solution of compound **35** was stirred at room temperature under nitrogen. Sulfur trioxide-pyridine complex (10.0 g, 62.8 mmol) was added in one portion, and the mixture was warmed to 80 °C for 45 minutes, after which TLC showed completion of the reaction. The solvent was removed *in vacuo*, and toluene (100 ml) was added to the residue. Again, the solvent was removed *in vacuo*. Ethyl acetate (200 ml) was added to the residue at 50 °C, and the suspension was cooled to approximately 25 °C and filtered. The flask and the filter

cake were washed with ethyl acetate (50 ml). A mixture of 25% sodium chloride (25 ml) and water (25 ml) was added to the slightly turbid filtrate at 20 °C. After a few minutes, a thick suspension was obtained. *t*-Butyl methyl ether (500 ml) was added, and the suspension was cooled to 0 °C, filtered, and washed with water (50 ml) and *t*-BuOMe (50 ml). The solid was dried (50 °C, 2 mm) to yield compound **36** as the sodium salt (17.3 g, 89% from compound **34**).

31. Alternate Method for Synthesis of Compound **37**

A 0.5 M sodium methoxide solution (10 ml, 5 mmol) was added to compound **36** (600 mg, 0.88 mmol) under nitrogen, and the reaction was brought to reflux for 3 hours. After leaving this mixture overnight at room temperature, the reaction was again heated at reflux for 5 hours. After evaporation, the residue was suspended in water (25 ml), neutralized (pH 7) with 1.5% trifluoroacetic acid, treated with brine, and extracted with methyl *t*-butyl ether. The aqueous layer was acidified to pH 2, saturated with sodium chloride, and extracted with tetrahydrofuran (6 x 100 ml, this entire volume is probably not necessary). The organic layers were dried with sodium sulfate, filtered, and evaporated to produce an oil, which was triturated with methyl *t*-butyl ether and collected by filtration to yield compound **37** (330 mg, 75%, mp 150-151°C): ^{1}H NMR (400 MHZ, THF-D_8): δ 4.11 (br q, 1H), 3.71 (br s, 1H), 2.4 - 1.0 (m, 27H), 1.02 (s, 3H), 0.94 (d, J = 6.5 Hz, 3H), 0.92 (d, J = 6.8 Hz, 3H), 0.89 (d, J = 6.7 Hz, 3H), 0.70 (s, 3H); ^{13}C NMR (400 MHZ, THF-D_8): δ 209.4, 84.3, 57.3, 51.5, 46.3, 45.1, 43.4, 41.0, 40.9, 40.2, 39.5, 38.8, 38.5, 37.1, 36.7, 32.2, 31.6, 29.2, 27.9, 26.0 24.6, 22.3, 19.4, 18.6, 18.5, 12.5, 10.7; MS (-FAB): 497.1 (M-1).

31b. Conditions for Synthesis of Compound **37**

A mixture of compound **36** (17.2 g, 27.5 mmol) in 1 M potassium hydroxide solution (150 ml, 150 mmol) in methanol under nitrogen was refluxed overnight. After evaporation of the solvent *in vacuo*, water (125 ml) and dichloromethane (125 ml) were added, and the suspension was cooled to 0 °C. After filtration, the solid was washed with water (3 x 30 ml) and dichloromethane (2 x 25 ml) and dried overnight (50 °C, 2 mm) to yield crude compound **37** as the potassium salt (12.5 g, 85%). Crude compound **37** (12.5 g, 23.3 mmol) was dissolved in a warm mixture of methanol (200 ml) and triethylamine (10 ml) and filtered to remove insolubles. The filtrate was concentrated on a rotary evaporator to approximately 75 ml, and *t*-BuOMe (100 ml) was added. After cooling to 0 °C, the suspension was filtered, washed with *t*-BuOMe (30 ml), and dried to give the potassium salt of compound **37** (10.4 g, 70%, mp 165-174 °C) as a white solid: ^1H NMR (400 MHZ, DMSO-D_6): ä 4.16 (m, 1H), 3.78 (br q, J = 5 Hz, 1H), 3.61 (m, 1H), 2.5 - 1.0 (m, 27H), 0.94 (s, 3H), 0.86 (d, J = 6 Hz, 3H), 0.81 (d, J = 7 Hz, 3H), 0.79 (d, J = 7 Hz, 3H), 0.63 (s, 3H); Anal. Calcd. for $C_{27}H_{45}O_6S$-0.77K-0.1Na-0.2H_20: C, 60.76; H, 8.57; H_2O, 0.68; K, 5.64; Na, 0.43. Found: C, 60.12; H, 8.21; H_2O, 0.66; K, 5.61; Na, 0.44; IR (KBr, cm^{-1}): 3436, 2928, 1708, 1470, 1390, 1208, 1051, 1038, 950, 812.

32. Preparation of Compound **38** (which corresponds to Compound 1436)

3 A molecular sieves (1 gram) were added to the clear colorless solution of compound **37** (16 mg, 0.032 mmol) and spermine (20 mg, 0.1 mmol, commercially available from Aldrich) in anhydrous methanol (3 ml). The reaction was stirred at room temperature under nitrogen for 12 hours, cooled to -78°C, and treated dropwise with sodium borohydride (1 pellet, 0.4 g, 10 mmol) in methanol (10 ml). This reaction mixture was stirred for 3 hours, treated with a mixture of water and methanol (10 ml each), warmed to room temperature, and then treated with 0.78% trifluoroacetic acid (TFA) solution until its pH reached the range of 4 - 5. The resulting mixture was filtered through a thin pad of Celite®, and the Celite® was washed with methanol and water (100 ml). Celite® is SiO_2 that is commercially available from Aldrich. The combined acidic washes were concentrated *in vacuo* at room temperature and then freeze-dried overnight to give a white solid. The Celite® cake was then washed with isopropyl amine/methanol/water (140 ml of 1:3:3), and the basic portion was evaporated to reduce its volume. This material was freeze-dried overnight to give a light brown solid. Both washes contained compound **38**, so they were combined and acidified to a pH of 3 with 0.78% TFA, filtered, and loaded onto a small HPLC column (1 cm diameter, see below). The reaction product was compound **38** (12.2 mg, 36%): ^{1}H NMR (400 MHZ, D_2O): δ 4.14 (m, 1H), 3.83 (m, 1H), 3.2 - 3.0 (m, 13H), 2.1-1.0 (m, 35H), 0.92 (m, 9H), 0.82 (s, 3H), 0.67 (s, 3H); ^{13}C NMR (400 MHZ, D_2O): δ 87.2, 68.0, 57.9, 56.0, 50.5, 47.4, 45.6, 44.9, 42.8, 41.9, 39.7, 37.5, 36.9, 36.7, 36.0, 35.8, 31.5, 31.1, 30.6, 28.3, 27.1, 24.8, 24.1, 23.6, 23.4, 23.1, 21.4, 19.2, 17.7, 12.1, 11.2; MS (-LD): 684 (M-1); Anal. Calcd. for $C_{37}H_{72}N_4O_5S$-3TFA-$2H_2O$: C, 48.58; H, 7.49; F, 16.08; N, 5.27; S, 3.02. Found: C, 48.49; H, 7.40; F, 16.16; N, 5.31; S, 3.05.

33.Purification of Compound **38** by HPLC

The crude material was dissolved in water (50 ml), cooled in an ice bath, and acidified with 1.5% TFA in water until its pH was 3. Initially, it was observed that one obtains a suspension as the pH drops, and then a solution is obtained at lower pH. This solution was loaded onto a Rainin reverse phase HPLC system (2.14 cm diameter, C18, 100 Å, 8μm) and eluted with A (water with 0.1% TFA) and B (acetonitrile with 0.1% TFA). The HPLC program was as follows: 10 min (0 - 10% B), 60 min (10-45% B), 10 min (45-80% B), 10 min (80% B). Pure product eluted in the 33 to 55 minute fractions, as determined by TLC (r_f: 0.1-0.2 in 6/3/1 CH_2Cl_2/MeOH/NH_4OH)(should evaporate plates under vacuum before eluting, and observe with ninhydrin stain after eluting), which was lyophilized to produce 1.20 grams of compound 1436 as a white powder (70%); $C_{37}H_{72}N_4O_5S$-3TFA-2.5H_2O, FW 1072.18).

34.Allylic Oxidation of Stigmasterol (Fig. 16B)

Stigmasterol (compound **50**, 150 g, 363 mmol) (obtainable from Aldrich) and N-hydroxypthalamide (60 g, 368 mmol) were added to a 3000 ml 3 neck round bottom flask. A 50/50 mixture of ethyl acetate/acetone (approximately 2500 ml) was added to the flask. The flask was equipped with a glass fritted air inlet and condenser and warmed to approximately 55°C with magnetic stirring. As the solution warmed, the stigmasterol and N-hydroxypthalamide dissolved. Dibenzoyl peroxide (approximately 250 mg) was then added to the reaction. Air was vigorously bubbled into the reaction with vigorous magnetic stirring, and the temperature of the reaction was maintained at 50-55°C throughout the course of the reaction. Additional 50/50 ethyl acetate/acetone solvent was added to the reaction as needed to replenish

that which was lost due to air flow through the system. The reaction was followed by TLC on silica gel (40% ethyl acetate in hexane) and judged to be complete after 48 hours. The reaction was worked up by adding the solution to cyclohexane (1000 ml) and allowing it to cool. The N-hydroxypthalamide was filtered off, and the remainder was removed by repetitive sodium carbonate washings until no orange coloration was observed. The organic layers were washed with brine and dried over $MgSO_4$. The solvent was removed *in vacuo*, and the sterol was dissolved in pyridine (500 ml). The pyridine solution was cooled to 0-4°C, and $CuCl_2$ (1 g) was added. The solution was stirred overnight, allowing the solution to warm to room temperature as the ice melted. The pyridine solution was then poured over an ice/water slurry (4000 ml), and the sterol precipitated. This solid was filtered, washed with 0.1 N HCl solution and distilled water, and then recrystallized from methanol (2X) to yield compound **60** (127 g, 298 mmol, 82%): mp 144°C; ^{1}H NMR (200 MHZ, $CDCl_3$): δ 5.71 (s, 1H), 5.26 - 4.95 (m, 2H), 3.69 (m, 1H), 1.20 (s, 3H), 1.02 (d, J = 6.5 Hz, 3H), 0.86 -0.78 (m, 9H), 0.70 (s, 3H); ^{13}C NMR (200 MHZ, $CDCl_3$): δ 202.5, 165.8, 137.9, 129.3, 125.6, 70.0, 54.4, 51.0, 49.8, 49.7, 45.2, 42.8, 41.6, 40.1, 38.4, 38.1, 36.2, 31.7, 30.8, 28.8, 26.2, 25.2, 21.2, 20.9, 18.8, 17.1, 12.1, 12.0; MS (FD): 426 (M^+); Anal. Calcd. for $C_{29}H_{46}O_2$: C, 80.63; H, 10.87. Found: C, 81.77; H, 11.04. (NOTE: 76.91 ppm was used as reference)

35.Lithium NH_3 Reduction of 7-Oxo Stigmasterol

Tetrahydrofuran (500 ml) was added to a 2000 ml 3 neck flask equipped with a dry ice condenser, 250 ml addition funnel, and magnetic stir bar. The condenser and a bath surrounding the flask were charged with dry ice acetone, and ammonia was collected to a total volume of

1200 ml. Lithium wire (2 g, 288 mmol) was added to the solution with vigorous stirring. Once the lithium was completely dissolved, a solution of compound **60** (25 g, 58.6 mmol) in tetrahydrofuran (100 ml) was added to the flask in a steady stream. After 1 hour, the reaction mixture was quenched by the addition of NH_4Cl and allowed to evaporate overnight. The resulting solid was dissolved in 500 ml toluene/1000 ml 1N HCl solution with vigorous stirring. After removal of the aqueous layer, the organic layer was washed with distilled water and brine, dried over $MgSO_4$, and evaporated. The residue was recrystallized from 2-propanol to yield compound **61** (19.4 g, 45.7 mmol, 78%): mp 149°C; ^{1}H NMR (200 MHZ, $CDCl_3$): δ 5.23 - 4.92 (m, 2H), 3.70 - 3.52 (m, 1H), 1.10 (s, 3H), 1.02 (d, J = 6.5 Hz, 3H), 0.86 - 0.78 (m, 9H), 0.70 (s, 3H); ^{13}C NMR (200 MHZ, $CDCl_3$): δ 212.0, 138.0, 129.3, 70.5, 55.1, 54.8, 51.1, 49.8, 48.8, 46.7, 46.0, 42.3, 40.1, 38.5, 37.7, 36.0, 35.9, 31.7, 30.9, 28.8, 25.2, 24.9, 21.7, 21.2, 20.9, 18.8, 12.1, 11.7; MS (FD): 428 (M^+): Anal. Calcd. for $C_{29}H_{48}O_2$: C, 81.25; H, 11.29. Found: C, 80.97; H, 11.20 (NOTE: 76.91 ppm was used as reference.).

36.K-Selectride® (potassium tri-*sec*-butylborohydride from Aldrich) Reduction of 7-Ketone

Compound **61** (10 g, 23.4 mmol) was dissolved in dry tetrahydrofuran (50 ml) in a 250 ml round bottom flask under argon. The flask was chilled to -20°C, and 1 M K-Selectride® solution (potassium tri-*sec*-butylborohydride from Aldrich) in tetrahydrofuran (51.6 ml, 51.6 mmol) was slowly syringed into the flask. The reaction was allowed to stir overnight, warming to room temperature as the ice melted. The reaction was cooled in an ice bath and quenched with a 30% H_2O_2 solution until the color disappeared and evolution of gas ceased. Toluene (250 ml) was added to the solution, and the organic layer was washed with distilled water, 1 N HCl

solution (2 x 250 ml), sodium bicarbonate solution, and brine. The organic layer was then dried, and the solvent was removed *in vacuo*. The resulting solid was then chromatographed on silica gel (elution with 60% ethyl acetate in hexane) to produce compound **62** (9.6 g, 22.4 mmol, 96%) as a white solid: mp 174°C; ^{1}H NMR (200 MHZ, $CDCl_3$): δ 5.22 - 4.92 (m, 2H), 3.82 (sharp m, 1H), 3.71 - 3.52 (m, 1H), 1.02 (d, J = 6.5 Hz, 3H), 0.86 - 0.81 (m, 12H), 0.68 (s, 3H); ^{13}C NMR (200 MHZ, $CDCl_3$): δ 138.1, 129.2, 71.0, 67.8, 55.8, 51.1, 50.5, 45.7, 42.4, 40.4, 39.4, 39.2, 37.6, 36.9, 36.6, 36.2, 35.4, 31.7, 31.2, 28.7, 25.2, 23.6, 20.9, 20.8, 18.8, 12.1, 11.9, 11.1; MS (FD): 430 (M^+); Anal. Calcd. for $C_{29}H_{50}O_2$: C, 80.87; H, 11.70. Found: C, 80.62; H, 11.76 (NOTE: 76.91 ppm was used as reference.).

37.Silver Carbonate on Celite® (SiO_2) Oxidation of 3B-ol

Silver carbonate on Celite® (SiO_2, available from Aldrich) was prepared by dissolving $AgNO_3$ (8.3 g, 49 mmol) in deionized water (250 ml) and adding Celite® (6.7 g, SiO_2 from Aldrich) to the solution. The solution was stirred vigorously to suspend the Celite® (SiO_2 from Aldrich). A large excess of a pH 11 carbonate buffer was slowly added to the slurry, and silver carbonate precipitated out onto the Celite® (SiO_2 from Aldrich) as a yellow-green solid. The solid was filtered, washed with deionized water, and dried in a foil covered vacuum desiccator overnight. The 3ß-hydroxy sterol **62** (7 g, 16.3 mmol) was dissolved in toluene (600 ml) in a 1000 ml round bottom flask equipped with a Dean Stark trap. The silver carbonate was added to the flask, and the solution was refluxed for 8 hours. The reaction was cooled and filtered through a short column of Florisil® (elution with ethyl acetate) to insure complete elution of the sterol. Florisil® is a magnesium silicate material that is commercially available from Aldrich.

The solvent was removed *in vacuo* to yield compound **63** (6.4 g, 15 mmol, 92%). The sterol was pure by TLC and NMR, but it was discolored due to elution of a trace of silver impurity from the Florisil® (magnesium silicate, available from Aldrich): mp 174 - 175°C; ^{1}H NMR (200 MHZ, $CDCl_3$): δ 5.23 - 4.92 (m, 2H), 3.84 (sharp m, 1H), 1.02 (d, J = 6.5 Hz, 3H), 1.00 (s, 3H), 0.93 - 0.82 (m, 9H), 0.69 (s, 3H); ^{13}C NMR (200 MHZ, $CDCl_3$): δ 211.6, 138.0, 129.3, 67.4, 55.8, 51.1, 50.4, 45.1, 44.0, 42.4, 40.4, 39.3, 39.2, 38.9, 38.0, 36.4, 35.6, 31.8, 28.8, 25.3, 23.6, 21.0, 18.9, 12.1, 11.9, 10.3; MS (FD): 428 (M^+); Anal. Calcd. for $C_{29}H_{48}O_2$: C, 81.25: H, 11.29. Found: C, 81.17; H, 11.49 (NOTE: 76.91 ppm was used as reference.).

38. Preparation of 7α-Benzoate (Compound **64**)

A cold (0°C) solution of compound **63** (5 g, 11.7 mmol) in pyridine (100 ml) was treated dropwise with benzoyl chloride (6.8 ml, 58.5 mmol). After the addition of 4-dimethyalaminopyridine (200 mg), the reaction mixture was allowed to warm to room temperature, stirred for approximately 8 hours, and then poured over ice and allowed to stand overnight. The resulting solution was filtered, leaving the sterol behind as a thick waxy solid. The sterol was dissolved in toluene, and washed with 1N HCl solution (2X) and sodium bicarbonate solution. The resulting organic layer was dried over $MgSO_4$, filtered, and evaporated. The residue was purified by chromatography on silica gel (gradient elution with ethyl acetate in toluene) to yield compound **64** (5.33 g , 10.1 mmol, 86%) as a white solid: mp 155°C; ^{1}H NMR (200 MHZ, $CDCl_3$): δ 8.04 - 7.99 (m, 2H), 7.63 -7.40 (m, 3H), 5.24 - 4.92 (m, 3H), 1.09 (s, 3H), 1.04 (d, J = 6.5 Hz, 3H), 0.85 - 0.70 (m, 12H); ^{13}C NMR (200 MHZ, $CDCl_3$): δ 210.8, 165.3, 137.8, 132.7, 130.4, 129.3, 129.2, 128.2, 71.0, 55.5, 50.9, 50.4, 46.8, 43.6, 42.3,

40.3, 40.1, 39.0, 38.3, 38.0, 37.8, 35.4, 33.2, 31.6, 28.5, 25.1, 23.4, 21.0, 20.9, 18.7, 12.0, 11.7, 10.3; MS (FD): 532 (M^+); Anal. Calcd. for $C_{36}H_{52}O_3$: C, 81.15; H, 9.84. Found: C, 80.98; H, 9.89 (NOTE: 76.91 ppm was used as reference.).

39. Preparation of 3-Dioxolane (Compound **65**)

The ketone **64** (4 g, 7.5 mmol) was dissolved in toluene (250 ml), and was treated with *p-toluenesulfonic acid (250 mg) and ethylene glycol (5 ml). The reaction mixture was heated to* reflux with the removal of water for 2 hours, and then allowed to cool. The reaction was treated with anhydrous sodium carbonate (2 g) and water. The organic layer was washed with sodium bicarbonate (2X), deionized water, and brine; dried over Na_2SO_4; and evaporated to yield compound **65** (4.1 g, 7.1 mmol, 95%) as a light yellow waxy solid: mp 74°C; ^1H NMR (200 MHZ, $CDCl_3$): δ 8.14 - 8.04 (m, 2H), 7.63 - 7.40 (m, 3H), 5.23 - 4.92 (m, 3H), 3.87 (m, 4H), 1.00 (d, J = 6.5 Hz, 3H), 0.89 (s, 3H), 0.81 - 0.70 (m, 12 H); ^{13}C NMR (200 MHZ, $CDCl_3$): δ 165.8, 138.0, 132.6, 130.9, 129.5, 129.2, 128.3, 108.9, 71.8, 64.0, 55.6, 51.1, 50.7, 47.1, 42.4, 40.4, 39.3, 38.5, 37.2, 37.1, 35.6, 35.4, 33.1, 31.7, 31.1, 28.6, 25.2, 23.6, 21.0, 18.8, 12.1, 11.8, 10.4; MS (FD): 576 (M^+); Anal. Calcd. for $C_{38}H_{56}O_4$: C, 79.12; H, 9.78. Found: C, 78.89; H, 9.75 (NOTE: 76.91 ppm was used as reference.).

40. Ozonolysis of Compound **65**

The sterol **65** (3.5 g, 6.1 mmol) was dissolved in 2/1 dichloromethane/ethanol (250 ml). The Welsbach apparatus was purged with oxygen at 7 psi (1 ml/min), and the water was turned on. The sterol solution was chilled in a dry ice ethanol bath. The ozonolyzer was set at 90 V and switched on. Ozone was bubbled into the magnetically stirred, chilled flask until a blue

coloration was observed. The power was switched off, and oxygen was bubbled into the flask until the color dissipated. Trimethyl phosphite (5 ml, 42 mmol) was added to the reaction pot, and the ice bath was removed to allow the reaction mixture to warm to room temperature. The solvent was removed *in vacuo* and maintained at high vacuum overnight to remove any remaining trimethyl phosphite. The resulting white solid was chromatographed on silica gel (gradient elution with ethyl acetate in toluene) to give compound **29** (2.7 g, 5.5 mmol, 90%) as a white solid identical to that prepared previously by NMR.

41.Preparation of Compound **41** (Fig. 17)

The following is an improved procedure over that published (Y. Umeda, M. Moriguchi, H. Kuroda, T. Nakamura, A. Fujii, H. Iinuma, T. Takeuchi, H. Umezawa, *J. of Antibiotics* **1987**, 1303 -1315). A flask containing 1,3-diaminopropane (2.5 kg, 33.72 mol) was stirred with a mechanical stirrer, cooled (- 6 °C), and treated with 4-bromobutyronitrile (1.00 kg, 6.76 mol) over 1.5 hours, maintaining the internal temperature at less than 0 °C. The reaction was allowed to stir in the cold bath for an additional 15 minutes. The cold bath was removed, and the reaction was allowed to stir without auxiliary temperature control for 1 hour. Isopropanol (11 L) was added in one portion to the reaction. The mixture was stirred for 15 minutes after the appearance of a precipitate, and then stored at 0 - 10 °C overnight. The solids were collected by filtration in a Buchner funnel lined with a polypropylene felt filter pad. The solids were washed with isopropanol (2 x 1.1 L). The combined filtrate was passed through an ion-exchange resin column.

The Dowex 1X8-100 ($^{-}$OH form) resin was prepared by combining Dowex 1X8-100 ($^{-}$Cl form, 2.2 kg) and aqueous 5 N NaOH (6 L) for one hour. The mixture was poured into a suitably sized chromatography column with a coarse glass frit. The resin was washed with 5 N NaOH (53 L). After washing with 44 L of 5 N NaOH, a small aliquot of the eluant, neutralized to pH 7 with HOAc, appeared hazy when aqueous 0.1 M $AgNO_3$ was added. The additional wash with 9 L of 5 N NaOH did not visibly improve clarity. The resin was washed with deionized water (6.6 L), at which time the pH of the eluant was 7. The eluant was clear after the addition of aqueous 0.1 M $AgNO_3$. The resin was washed with isopropanol (11 L), and the column was ready for use.

After the entire filtrate had passed through the column, the column was washed with isopropanol (14 L). A small aliquot of the combined eluant, when neutralized to pH 7 with HOAc, was clear after the addition of aqueous 0.1 M $AgNO_3$. The combined eluant was concentrated to a weight of 1.39 kg using a water aspirator and a bath at 45 - 50 °C. Molecular sieves (100 g, 3 A) were added to the residue, which was stored at 0 - 10 °C overnight. The sieves were removed by filtration, and the filtrate was distilled under reduced pressure in a 2 L flask equipped with overhead stirring, a thermometer, and a distillation head with a short Vigreaux column. Fractions distilling at less than 114 °C (0.6 mm) were collected and discarded (627 g). Two product fractions were collected containing pure compound **41**: fraction 4 (108 g) distilled at 114 - 115 °C (0.6 mm) and fraction 5 (591 g, total = 699 g, 73%) distilled at 110 - 112 (0.5 mm): ^{1}H NMR (400 MHZ, $CDCl_3$): ä 2.71 (t, J = 6.5 Hz, 2H), 2.68 (t, J = 6.5 Hz, 2H), 2.61 (t, J = 7 Hz, 2H), 2.39 (t, J = 7 Hz, 2H), 1.75 (p, J = 7 Hz, 2H), 1.56 (p, J = 7 Hz, 2H); ^{13}C

NMR (100 MHZ, $CDCl_3$): ä 119.9, 48.1, 47.7, 40.5, 33.8, 25.8, 15.0; MS ($^+$FAB): 142 (M^+, 100); IR (neat, cm^{-1}): 3280, 2930, 2244, 1592, 1470, 1128, 830; Anal. Calcd. for $C_7H_{15}N_3$: C, 59.54; H, 10.71; N, 29.76. Found: C, 58.60, H, 10.52, N, 28.86.

42. Preparation of Compound **42** (Squalamine)

The polyamine **41** (8.00 g, 56.7 mmol) was dissolved in anhydrous methanol (650 ml) at *room temperature, and trimethyl orthoformate (50 ml, 457 mmol) was added. Steroid* **37** *(10.0 g,* 18.7 mmol) was added, and the reaction mixture was stirred for 18 hours. The reaction mixture was cooled to -74 °C, treated with sodium borohydride (1.06 g, 28.0 mmol) over one minute, and stirred for 3.5 hours at -74 °C. The reaction was allowed to warm to room temperature and was concentrated at 31 °C under a water aspirator vacuum. The crude product was dissolved in 100% ethanol *(290* ml), purged with nitrogen, and acidified to pH *1-2 with neat trifluoroacetic* acid. Platinum oxide (1.00 g) was added, and the mixture was shaken on a Parr apparatus (40 psi) for 18 h. The reaction mixture was filtered through paper, which was washed with methanol (620 ml). The filtrate was evaporated and then dissolved in 50% ethanol in water.

A propyl sulfonic acid ion exchange column was prepared by suspending 80 g of resin in 10% isopropanol (IPA) in water to form a slurry and by adding 200 ml of 10% IPA to the column, followed by the slurry. At least five column volumes of 10% IPA were eluted through the column at a flow rate of 40 ml/min. The column was washed with 0.05% trifluoroacetic acid (TFA) in 50% ethanol in water (150 ml) at 20 ml per min. The sample from above was loaded (in two batches), and the eluant was collected. The column was washed with two column volumes of 0.05% TFA in 50% ethanol in water and two column volumes of 0.05% TFA in 10%

IPA. The column was then eluted with 4.5 M KOAc/10% IPA (pH 5), and the fractions were collected (150 ml each). Fractions that contained squalamine by TLC (6 : 3 : 1, dichloromethane : methanol : ammonium hydroxide) were combined.

The crude material from ion exchange was purified on a 25 x 5 cm YMC ODS-AQ C18 reversed phase column. The eluant from PSA was diluted with four volumes of deionized water. The column was loaded with squalamine and eluted with four column volumes of buffer A (0.05% TFA in 1% acetonitrile in water). Then the column was eluted with the following gradient of buffers A and B (0.05% TFA in 1% water in acetonitrile)(Detector: UV ë = 200 nm):

min	ml/min	% A	% B
0	0	100	0
1:00	100	100	0
10:00	100	75	25
60:00	100	60	40
70:00	100	40	60
80:00	100	20	80
90:50	100	20	80
98:00	100	100	0
99:00	0	100	0

Between 28 and 60 minutes (30 - 40% B), fractions were collected (50 ml). All fractions were examined by TLC, and the early and late fractions were analyzed by analytical HPLC with

ortho-phthalaldehyde (OPA) derivitization, a reverse phase column, and fluorescence detection. Fractions that were > 95% pure were combined to afford 97% pure compound **42** (10.3 g, 60% yield) as the TFA salt (approximate FW 910), that was identical to natural squalamine by anal. HPLC (OPA method); ^{1}H NMR (CD_3OD, 400 MHZ): ä 4.12 (br q, 1H), 3.76 (br s, 1H), 3.2 - 2.9 (m, 9H), 2.1 - 1.0 (m, 33H), 0.94 - 0.90 (m, 9H), 0.84 (s, 3H), 0.67 (s, 3H); and ^{13}C NMR (CD_3OD, 100 MHZ): ä 86.7, 68.4, 59.2, 57.7, 51.8, 46.8, 46.0, 43.9, 43.0, 41.2, 40.1, 38.7, 38.0, 37.8, 37.5, 37.0, 32.7, 32.2, 32.1, 29.5, 28.3, 26.1, 25.7, 24.7, 24.6, 24.3, 22.3, 19.6, 18.6, 18.3, 12.6, 11.7.

In describing the invention, applicant has stated certain theories in an effort to disclose how and why the invention works in the manner in which it works. These theories are set forth for informational purposes only. Applicants do not wish to be bound by any specific theory of operation.

While the invention has been described in terms of various specific preferred embodiments and specific examples, those skilled in the art will recognize that various changes and modifications can be made without departing from the spirit and scope of the invention, as defined in the appended claims.

CLAIMS:

1. A method for stereoselectively reducing an unsaturated alkyl ketone substituent attached to a fused ring base, comprising:

reacting the unsaturated alkyl ketone with a chiral oxazaborolidine reagent to stereoselectively reduce the unsaturated alkyl ketone to an unsaturated alkyl alcohol.